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Free Radical Scavenging Activity of NokJoongTang Prepared from Antler and Various Oriental Medicinal Materials

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녹용과 한약재료로 조제된 녹중탕의 유리기 소거 활성

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Abstract

Two different NokJoongTangs were prepared by boiling antler and various oriental medicinal materials. The phenolic contents and free radical scavenging effects against DPPH(1,1-diphenyl-2-picrylhydrazyl) radical and hydroxyl radical of the NokJoongTangs which are used as the health supplement foods, were investigated. NokJoongTang-II prepared dried antler with various oriental medicinal materials, had higher phenolic content than that of NokJoongTang-I prepared from raw antler with the same materials. In addition, NokJoongTang-II exhibited higher scavenging activities than these of NokJoongTang-I against DPPH radical and hydroxyl radical. These results indicate that free radical scavenging activity of NokJoongTang showed a difference by using raw and dry antler for NokJoongTang preparation, and that the findings also show a positive correlation between radical scavenging activity and total phenolic contents.

Key words : NokJoongTang, phenolic content, antler, radical scavenging activity

Introduction

The antioxidant is defined as any substance that significantly delays or inhibits oxidation of that substrate when present at low concentrations compared to that of an oxidizable substrate. Many researches have shown the implication of oxidative and free radical-mediated reactions in degenerative processes related to aging (Ames *et al.*, 1993; Harman, 1995) and diseases such as cancer, coronary heart disease, and neurodegenerative disorders such as Alzheimer's disease (Frlich and Riederer, 1995; Gey, 1990; Diaz *et al.*, 1997). Prooxidants and free radicals were produced during normal cell aerobic respiration, and the free radicals are trapped and destroyed by antioxidant enzymes such as catalase, superoxide dismutase, and glutathione peroxidase. However, overproduction of free radicals reduces the level of the antioxidant enzymes and induces A, C and E avitaminosis (Ellnain-Wojtaszek *et al.*, 2003). Therefore, antioxidants are important

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for body protection against oxidative stress. Many synthetic antioxidants and natural antioxidants from various sources have been reported. However, the use of synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tert-butylhydroquinone (TBHQ), and propyl gallate is under stric regulation in food products due to their potential adverse effects on health (Hettiarachchy *et al.*, 1996). Therefore, research for natural antioxidants as alternatives to synthetic ones is of great interest among researchers.

In Korea, NokJoongTang, which is prepared from boiled water-soluble extract of antler and various oriental medicinal materials is used a lot as a health supplement food for recovery of vigor, riddance of stress, improvement of arthropathy, and protection of vertigo and asthma and so on (Shin, 2002). However, little information on the free radical scavenging activity from NokJoongTang was available until now. In the present study, two different NokJoongTangs were prepared with antler and various oriental medicinal materials. In addition, phenolic contents and free radical scavenging activities of the NokJoongTangs against hydroxyl radical and DPPH radical were investigated.

Materials and Methods

Materials

Antler and deer meat was obtained from our nokyong research center, and wolfiporia cocos, angelica, cnidium officinale, steamed rehmannia root, paeony, astragalus membranaceus, cinnamon, and licorice were purchased from a local oriental medical market. 5,5-Dimethyl-1-pyrrolin N-oxide (DMPO), and DPPH were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other reagents were of the highest grade commercially available.

Preparation of NokJoongTang-I and II

NokJoongTang-I and II were prepared by boiling the mixture of raw antler (37.5 g; NokJoongTang-I) and dried antler (37.5 g; NokJoongTang-II), deer meat (1.2 kg), wolfiporia cocos (100 g), Atracylodes rhizome white (100 g), angelica (100 g), cnidium officinale (100 g), steamed rehmannia root (100 g), paeony (100 g), astragalus membranaceus (100 g), cinnamon (100 g), and licorice (83 g) for different times. NokJoongTang-I was boiled for 12 h, and NokJoongTang-II was boiled for 6 h.

Total Phenolic Assay

Total phenolic contents were determined using the method described by Chandler and Doodds (1983) with a little modification. The mixture of 1.0 mL of NokJoongTang, 1.0 mL of ethanol, 5.0 mL of distilled water, and 0.5 mL of 50% Folin-Ciocalteu reagent were mixed, and then the solution was allowed to react for 5 min. One milliliter of 5% Na_2CO_3 was added, and placed in the dark place for 1 h after the mixture. The total phenolic contents were calculated by measuring the absorbance of each sample at 725 nm using gallic acid as a calibration standard. All samples were assayed in triplicate, and the data were expressed by means.

DPPH Radical Assay

DPPH radical scavenging activity was measured using the method described by Nanjo *et al.* (1996). An ethanol solution of each sample or ethanol itself as control (60 μL) was added to 60 μL of DPPH (60 μmol/L) in ethanol solution. After mixing vigorously for 10 s, the solutions were then transferred into a 100 μL Teflon capillary tube and fitted into the cavity of the ESR(electro spin resonance) spectrometer. The spin adduct was measured on an ESR spectrometer exactly 2 min later. Measurement conditions were central field 3475 G, modulation frequency 100 kHz, modulation amplitude 2 G, microwave power 5 mW, gain 6.3×10^5 and temperature 298 K.

Hydroxyl Radical Assay

Hydroxyl radicals were generated by Fenton reaction, and reacted rapidly with nitrone spin trap DMPO: the resultant DMPO-OH adducts were detectable with an ESR spectrometer (Rosen and Rauckman, 1984). Phosphate buffer solution (pH 7.4) with 0.3 M DMPO 0.2 mL, 10 mM $FeSO_4$ 0.2 mL, 10 mM H_2O_2 0.2 mL and sample dissolved in the buffer was mixed, after the ESR (electron spin resonance spectrometer; JEOL, Tokyo, Japan) spectrum was recorded. The conditions were set as follows; central field 3475 G, modulation frequency 100 kHz, modulation amplitude 2 G, microwave power 1 mW, gain 6.3×10^5 and temperature 298 K.

Results and Discussion

Antler, as an animal source of the folk medicine, has been used in therapy of neurosis, enriching the vital energy, nursing the blood, strengthening the kidney and prolonging life for thousands of years. Recently, many researchers reported its various biological effects such as anti-inflammatory effects (Zhang *et al.*, 1992), anti-whiplash activities as anti-stress activities (Takikawa *et al.*, 1972), anti-aging activities (Wang *et al.*, 1988), and so on. However, there have been no reports discussed on the total phenolic content and free radical effect of antlers and NokJoongTang, which is prepared by boiling the mixture of antler and various oriental medicinal materials. In the present study, the total phenolic contents of the NokJoongTang-I and II are shown in Fig. 1. NokJoongTang-II (7.55 mg/100 g) had higher phenolic content than that of NokJoongTang-I (6.26 mg/100 g). In the recent years, several studies have been reported on the relationships between phenolic content and antioxidant activity. Some researchers found a correlation between the phenolic content and antioxidant activity, while others found no such relationship. Velioglu *et al.* (1998) reported a strong relationship between total phenolic content and antioxidant activity in selected fruits, vegetables and grain products. No correlation between antioxidant activity and phenolic content was found in the study by Kähkönen *et al.* (1999) on some plant extracts containing phenolic compounds. Therefore, we studied total phenolic contents and free radical scavenging activity of NokJoongTang-I and II to investigate their relationship. The DPPH radical signal of NokJoongTang-I is shown in Fig. 2, and a dose-response relationship was found in the DPPH radical scavenging activity; the activity increased as the concentration increased for each individual NokJoongTang. It was observed that 0.06, 0.13, 0.25, and 0.5 mg/mL of NokJoongTang-I scavenged 38.2%, 51.8%, 81.5% and 96.2%, respectively (Fig. 3). In addition, NokJoongTang-II exhibited

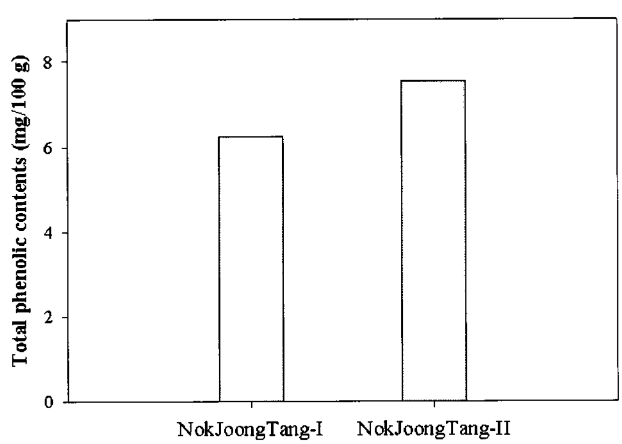

Fig. 1. Total phenolic contents of NokJoongTang-I and II. Each value is the mean of triplicate experiments.

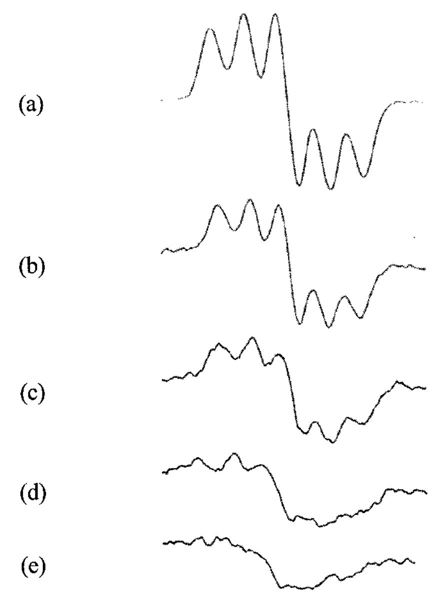

Fig. 2. ESR spectra obtained in a methanol solution of 30 μmol/L DPPH treated with various concentrations of NokJoongTang-I. (a), control (0 mg/mL); (b), 0.06 mg/mL; (c), 0.13 mg/mL; (d), 0.25 mg/mL; (e), 0.5 mg/mL.

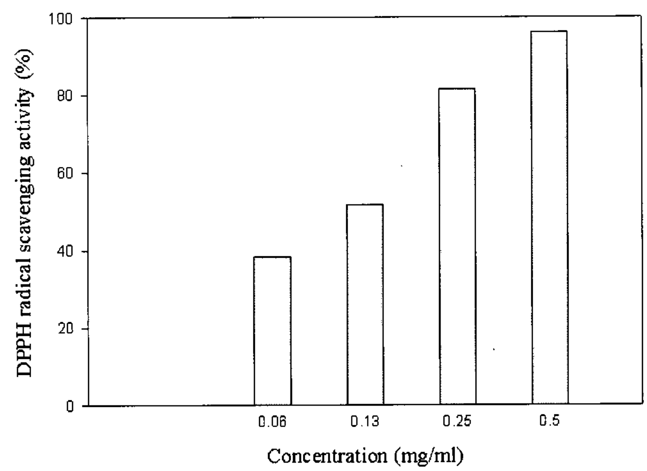

Fig. 3. DPPH radical scavenging activity of NokJoongTang-I. Each value is the mean of triplicate experiments.

the DPPH radical scavenging activity although NokJoongTang-II was a little higher activity than that of NokJoongTang-I (Fig. 4). Generally, DPPH signals decrease when the odd electron of DPPH radical is paired. The results indicate that NokJoongTang was found to possess DPPH radical scavenging activity by pairing the odd electron of DPPH radicals. The involvement of free radicals, especially their increased production, appears to be a feature of most, if not all human disease, including cardiovascular disease and cancer (Deighton *et al.*, 2000). Therefore, such antioxidants from NokJoong-Tang may be particularly important in fighting these diseases

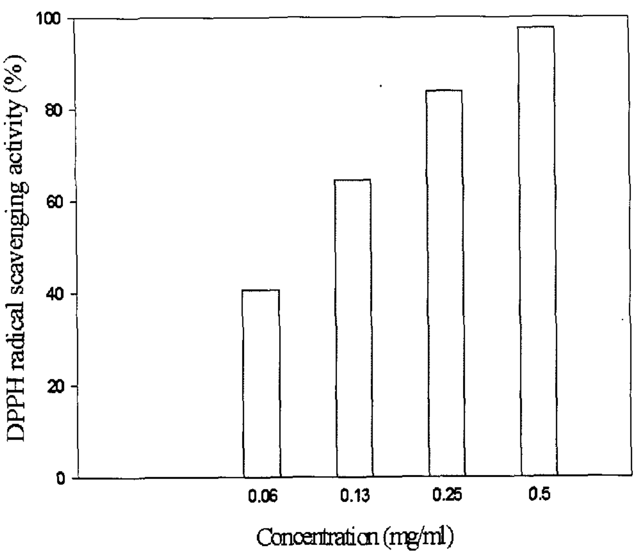

Fig. 4. DPPH radical scavenging activity of NokJoongTang-II. Each value is the mean of triplicate experiments.

by conferring protection against free radical damage to cellular DNA, lipids and proteins. Hydroxyl radicals generated in the Fe^{2+}/H_2O_2 system were trapped by DMPO, forming a spin adduct detected by ESR spectrometer, and the typical 1:2:2:1 ESR signal of the DMPO-OH adduct was observed as shown in Fig. 5. The hydroxyl radical scavenging activity of NokJoongTang-I was dose-dependent, and it was observed that 0.25 mg/mL, 0.5 mg/mL, and 1.0 mg/mL of NokJoong-Tang-I scavenged 22.48%, 38.76%, and 53.57%, respectively (Fig. 6). Additionally, NokJoongTang-II against hydroxyl radical scavenged 39.5%, 53.6%, and 63.6% at the same concentration, respectively (Fig. 7). As these results, NokJoong-Tang-I and II were prepared from raw and dry antler with the same oriental medicinal materials, and free radical scavenging of NokJoongTang-II exhibited higher than that of NokJoong-Tang-I against DPPH radical and hydroxyl radical. This result suggests that NokJoongTang extracted from raw and dry antler with various oriental medicinal materials show different free radical scavenging activity. In addition, These results also suggest that the findings show a relationship between free radical scavenging activity and total phenolic contents. Free radicals with the major species of reactive oxygen species (ROS) are unstable and react readily with other groups or substances in the body, resulting in cell damage and hence human disease (Halliwell and Gutteridge, 1989). It is generally considered that the inhibition of lipid peroxidation by an antioxidant may be due to the free radical scavenging activity. Lipids of biological membranes, especially those in

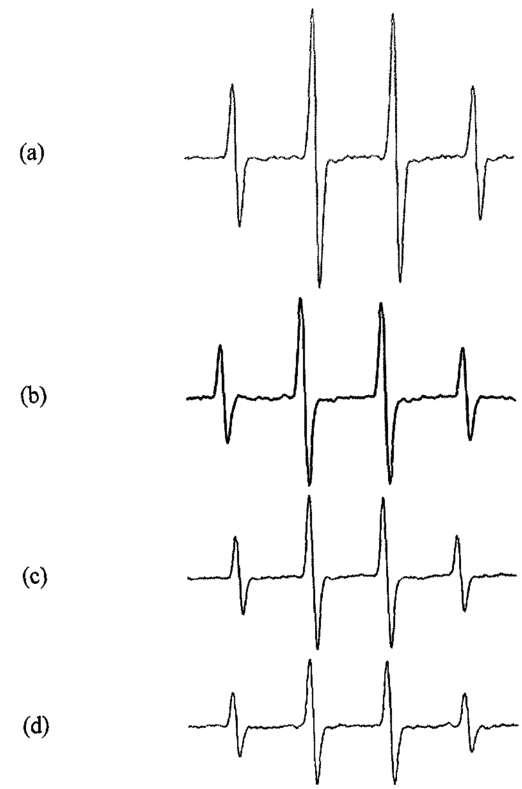

Fig. 5. ESR spectra obtained in Fenton reaction system treated with various concentrations of NokJoongTang-I. (a), control (0 mg/mL); (b), 0.25 mg/mL; (c), 0.5 mg/mL; (d), 1.0 mg/mL.

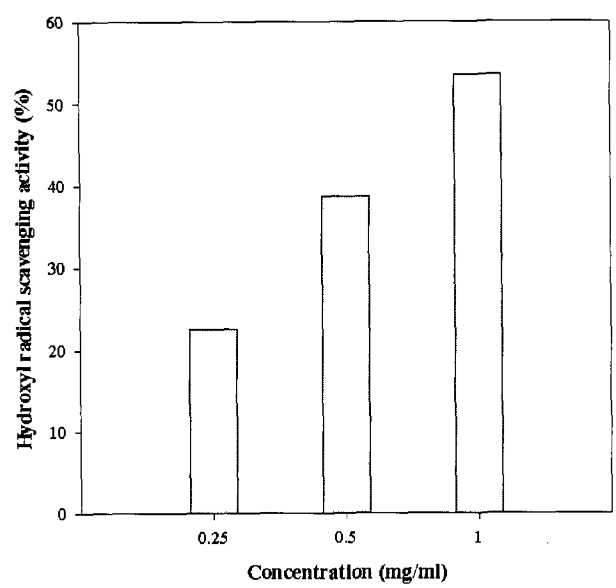

Fig. 6. Scavenging effects of NokJoongTang-I on hydroxyl radical obtained in Fenton reaction system at various concentrations. Each value is the mean of triplicate experiments.

the spinal cord and brain containing highly oxidizable polyunsaturated fatty acids, are particularly affected (Braughler

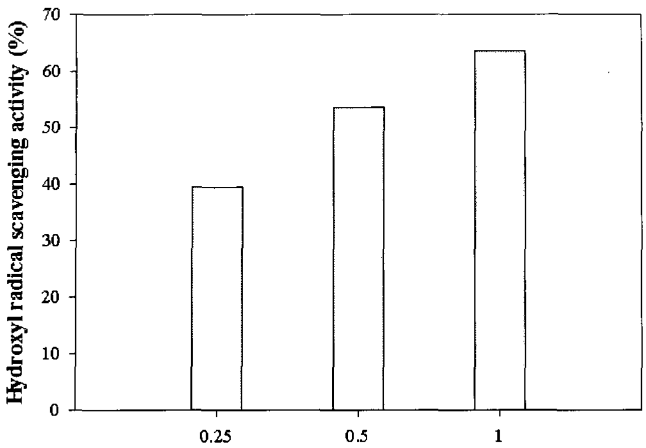

Fig. 7. Scavenging effects of NokJoongTang-II on hydroxyl radical obtained in Fenton reaction system at various concentrations. Each value is the mean of triplicate experiments.

and Hall, 1989). Moreover, the brain contains considerable amounts of prooxidant transition metal ions and utilizes a lot of oxygen. These properties set the stage for ROS generation and the subsequent acute cellular injury. Many researchers reported that several natural antioxidants have already been isolated from different kinds of plant materials, such as oil seeds, cereal crops, vegetables, fruits, leaves, roots, spices, and herbs (Ramarathnam *et al.*, 1995). In addition, antioxidant compounds have been identified in the seeds of citrus (Alessandra *et al.*, 1998), grape (Jayaprakasha *et al.*, 2001), mango (Puravankara *et al.*, 2000), canola (Naczk *et al.*, 1998), egg yolk (Park *et al.*, 2001), chitooligosaccharide (Park *et al.*, 2003), and seaweed (Park *et al.*, 2004). In summary, two different NokJoongTangs were prepared from antler and various oriental medicinal materials, and their free radical scavenging activities were identified on DPPH radical and hydroxyl radical using ESR spectroscopy. NokJoongTang-II prepared dried antler and oriental medicinal materials showed higher radical scavenging effects on DPPH and hydroxyl radical, compared to those of NokJoongTang-I prepared from raw antler and the same materials, and the activity depended on their concentrations. These results indicate that the conditions of antler shows the different biological activity of antler. Therefore, further study is needed to investigate biological properties of antler prepared by various dry conditions. In addition, findings show a positive correlation between radical scavenging activity and total phenolic contents.

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