Prev. Nutr. Food Sci. 2013;18(1):30-37
http://dx.doi.org/10.3746/pnf.2013.18.1.030
pISSN 2287-1098 · eISSN 2287-8602

Antioxidant and Neuronal Cell Protective Effects of Columbia Arabica Coffee with Different Roasting Conditions

Ji Hee Jeong[1], Hee Rok Jeong[2], Yu Na Jo[1], Hyun Ju Kim[1], Uk Lee[3], and Ho Jin Heo[1]

[1]Division of Applied Life Sciences, Institute of Agriculture and Life Science, Gyeongsang National University, Gyeongnam 660-701, Korea
[2]Pacificpharma Corporation, Gyeonggi 456-370, Korea
[3]Department of Special Purpose Trees, Korea Forest Research Institute, Gyeonggi 441-847, Korea

ABSTRACT: *In vitro* antioxidant activities and neuronal cell protective effects of ethanol extract from roasted coffee beans were investigated. Colombia arabica coffee (*Coffea arabica*) green beans were roasted to give medium (230°C, 10 min), city (230°C, 12 min) and french (230°C, 15 min) coffee beans. Total phenolics in raw green beans, medium, city and french-roasted beans were 8.81±0.05, 9.77±0.03, 9.92±0.04 and 7.76±0.01 mg of GAE/g, respectively. The content of 5-O-caffeoylquinic acid, the predominant phenolic, was detected higher in medium-roasted beans than others. In addition, we found that extracts from medium-roasted beans particularly showed the highest *in vitro* antioxidant activity on ABTS radical scavenging activity and FRAP assays. To determine cell viability using the MTT assay, extracts from medium-roasted beans showed higher protection against H_2O_2-induced neurotoxicity than others. Lactate dehydrogenase (LDH) leakage was also inhibited by the extracts due to prevention of lipid peroxidation using the malondialdehyde (MDA) assay from mouse whole brain homogenates. These data suggest that the medium-roasting condition to making tasty coffee from Columbia arabica green beans may be more helpful to human health by providing the most physiological phenolics, including 5-O-caffeoylquinic acids.

Keywords: *Coffea arabica*, coffee, neurotoxicity, roasting condition, 5-O-caffeoylquinic acid

INTRODUCTION

Reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), singlet oxygen, superoxide anion radical ($O_2\cdot^-$) and hydroxyl radical ($OH\cdot^-$) are generated from the autoxidation of lipids, as well as reactive nitrogen species (RNS) (1). Formations of these excess ROS and RNS by UV irradiation, smoking and drug metabolism are likely to damage several cellular components such as lipids, proteins, nucleic acids and DNA through oxidation or nitration processes (2). In addition, these reactive oxygen species cause inflammation or lesions on various organs, and are associated with various degenerative diseases, including cancer, aging, arteriosclerosis and neurodegenerative diseases (3,4). Alzheimer's disease (AD) is one of the most serious threats to human health in aged societies of developed countries. In particular, AD, one of the major neurodegenerative diseases, is characterized by loss of memory and cognition. After amyloid plaque formation, inflammation and oxidative stress further enhance the degeneration of neurons. Accumulation of intracellular H_2O_2 induces the peroxidation of membrane lipids and apoptotic cell death by activation of caspases (5,6). However, some phytochemicals from natural plant sources like fruits and vegetables may reduce the risk of AD because of their antioxidative properties diminishing oxidative insults (7).

Coffee has been one of the most popular beverages all over the world, and its consumption continues to increase due to its physiological effects as well as its pleasant taste and aroma. In addition, coffee is one of the most important food commodities both for producers, in tropical and subtropical areas with coffee as their main agricultural export product, and for manufacturers, which are mainly located in Europe and North America where coffee is roasted, mixed and packed. Due to its large diffusion and high market value, coffee is subject to adulteration throughout its production chain (8).

Generally, coffee has been reported to have beneficial effects on degenerative diseases including cancer (9), cardiovascular disorders (10), obesity and diabetes (11,12). Coffee contains phenolic acids including caffeic acid,

Received: January 15, 2013; **Accepted:** March 11, 2013

Correspondence to: Ho Jin Heo, Tel: +82-55-772-1907, E-mail: hjher@gnu.ac.kr

Copyright © 2013 by The Korean Society of Food Science and Nutrition. All rights Reserved.
This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/3.0) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

ferulic acid, and vanillic acid (13), and 5-O-caffeoylquinic acid as the predominant phenolic compound, measuring on average 100 mg per cup of coffee (14). Although roasted coffee has physiological activities because of their phytochemicals, little is known about the difference in physiological activities due to various roasting conditions for coffee beans. In addition, many complex physical and chemical changes take place during roasting from the obvious change in color from green to dark brown, to the major compositional changes such as decreases in protein, amino acids, arabinogalactan, reducing sugars, trigonelline, 5-O-caffeoylquinic acid, sucrose, and water, and the formation of melanoidins (15).

The objective of this experiment was to investigate the effects of coffee beans with various roasting conditions against oxidative stress-induced neurotoxicity.

MATERIALS AND METHODS

Materials

Folin-Ciocalteu's phenol reagent, 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt (ABTS), potassium persulfate, 2,4,6-tripyridyl-S-triazine (TPTZ), trichloroacetic acid (TCA), thiobarbituric acid (TBA), vitamin C, α-tocopherol, catechin, 2-[4-(2-hydroxyethyl) piperazin-1-yl]ethanesulfonic acid (HEPES), sodium bicarbonate, penicillin, streptomycin, 5-O-caffeoylquinic acid, syringic acid, epigallocatechin gallate, ferrous sulfate ($FeSO_4$), hydrogen peroxide (H_2O_2), dimethyl sulfoxide (DMSO), 3-[4,5-dimethythiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) assay kit, lactate dehydrogenase (LDH) assay kit and all solvents used were of analytical grade and purchased from Sigma Chemical Co. (St. Louis, MO, USA). RPMI 1640 medium and fetal bovine serum were obtained from Gibco BRL (Grand Island, NY, USA).

Sample preparation

Colombian arabica coffee (*Coffea arabica*) beans were purchased from the local barista institute in Jinju of Korea, in December 2010. The sample was roasted for 10, 12 and 15 min at 230°C to give medium-, city- and french-roasted coffee beans, respectively. Roasted samples (2 g) were suspended and extracted with 100 mL of 70% ethanol at 70°C for 2 hr. The extracts were frozen, lyophilized and placed in a glass bottle for storage (−20°C) until further use.

Neuronal cell culture

Neuronal PC12 cell line was derived from a transplantable rat pheochromocytoma. The cells respond reversibly to nerve growth factor (NGF) by induction of the neuronal phenotype. Cells (KCLB 21721, Korea Cell Line Bank, Seoul, Korea) were propagated in RPMI 1640 medium containing 10% fetal bovine serum, 50 units/mL penicillin and 100 μg/mL streptomycin.

ABTS radical scavenging activity

2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) was dissolved in water to make a concentration of 7 mmol/L. ABTS was produced by reacting the ABTS stock solution with 2.45 mM potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12∼16 hr before use. For the study of samples, the ABTS stock solution was diluted with phosphate-buffered saline (5 mM/L, pH 7.4) to an absorbance of 0.70 at 734 nm. After the addition of 980 μL of diluted ABTS to 20 μL of sample, the absorbance reading was taken 5 min after the initial mixing (16) and the percent ABTS scavenging activity is calculated as:

$$\% \text{ ABTS scavenging activity} = [(\text{control absorbance} - \text{ample absorbance})/(\text{control absorbance})] \times 100$$

Ferric reducing antioxidant power (FRAP)

To measure total antioxidant activity, the FRAP assay, developed by Jeong et al. (16), was used. In short, 1.5 mL of working, pre-warmed 37°C FRAP reagent (10 volumes of 300 mM sodium acetate buffer (pH 3.6), 10 mM 2,4,6-tripyridyl-S-triazine in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$) was mixed with 50 μL of test samples and standards. The mixture was vortexed and absorbance at 593 nm was read against a reagent blank at a predetermined time after sample-reagent mixing. The test was performed at 37°C and a 0∼4 min reaction time window was used.

Malondialdehyde (MDA) assay using mouse whole brain homogenates

The MDA assay was carried out to the method described by Chang et al (17). The brain of young adult male ICR mice were dissected and homogenized in ice-cold Tris-HCl buffer (20 mM, pH 7.4) to produce a 1/10 homogenate. The homogenate was centrifuged at 12,000×*g* for 15 minutes at 4°C. 1 mL aliquots of the supernatant were incubated with the test samples in the presence of 10 μM $FeSO_4$ and 0.1 mM vitamin C at 37°C for 1 hr. The reaction was terminated by addition of 1.0 mL TCA (28%, w/v) and 1.5 mL TBA (1%, w/v) in succession, and then the solution was heated at 100°C. After 15 minutes, the color of the MDA-TBA complex was measured at 532 nm. (+)-Catechin, a well-known antioxidant, was used as a positive control. The inhibition ratio (%) was calculated as follows:

$$\% \text{ inhibition} = [(\text{absorbance of control} - \text{absorbance of sample})/\text{absorbance of control}] \times 100$$

Determination of cell viability

[3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium-bromide] (MTT) reduction assay was determined using the *in vitro* toxicology assay kit (TOX-1, Sigma Co.). Neuronal PC12 cells were plated at a density of 10^5 cells/well in 96-well plates in 100 μL of RPMI. The cells were pre-incubated with various extracts obtained from roasted coffee for 48 hr before the cells were treated either with or without 200 μM H_2O_2 for 2 hr. The amount of MTT formazan product was determined by measuring absorbance using a microplate reader (680, Bio-Rad, Tokyo, Japan) at a test wavelength of 570 nm and a reference wavelength of 690 nm (17).

Neuronal PC12 cells were precipitated by centrifugation at 250×*g* for 4 min at 4°C, 100 μL of the supernatants were transferred into new wells, and lactate dehydrogenase (LDH) was determined using the *in vitro* toxicology assay kit (TOX-7, Sigma Co.). Damage of the plasma membrane was evaluated by measuring the amount of the intracellular LDH enzyme released into the medium (18).

Determination of total phenolics

Total phenolics were determined by spectrophotometric analysis (16). The standard curve for total phenolics was made using 5-O-caffeoylquinic acid standard solution (0∼100 mg/L). Total phenolics in roasted coffee were expressed as milligrams of gallic acid equivalents per gram (mg GAE/g) of sample.

Quantification by HPLC

Phenolics in extracts obtained from roasted coffee beans were measured at 280 nm by a photo diode array detector (Ultimate 3000 series, Dionex, Sunnyvale, CA, USA). Separation was achieved with a Shiseido C_{18} column (250 mm×4.6 mm ID×5 μm, Shiseido Co., Tokyo, Japan). The elution solvents were (A) 0.01 M potassium phosphate buffer adjusted to pH 3.0 by phosphoric acid and (B) methanol (99.9% concentrated). The solvent gradient elution program used was as follows: initial 90% (A), hold for 9.5 min; linear gradient to 68% (A), 3.5 min; linear gradient to 67% (A), 17 min; linear gradient to 20% (A), 1 min; linear gradient to 90% (A), 1 min, and hold for 10 min. The flow rate was 1.5 mL/min. Phenolics were identified by comparing their retention time (RT) values to UV spectra of known standards and quantified by peak areas from the chromatograms. All analyses were run in triplicate and mean values were calculated. Content of phenolic compounds was expressed in mg/g extract.

Statistical analysis

All data were expressed as mean±SD. Each experimental set was compared with one-way analysis of variance (ANOVA) and Duncan's multiple-range test ($p<0.05$) using SAS program (SAS Institute Inc., Cary, NC, USA).

RESULTS AND DISCUSSION

ABTS radical scavenging activities and FRAP

The reduction capability of ABTS induced by antioxidants was determined by the decrease in its absorbance at 734 nm. A positive ABTS test suggests that the extracts were free radical scavengers. The scavenging effect of extracts obtained from roasted coffee and vitamin C on ABTS radicals were compared. The extracts had significant ABTS radical scavenging effects with increasing concentrations in the range of 125∼1,000 mg/mL. A 1,000 μg/mL concentration of the extracts (green, medium, city and french) exhibited 46.99±5.40%, 59.85±1.98%, 55.27±0.08% and 56.5±1.22% inhibitions, respectively (Fig. 1A). However, when compared with that of vitamin C as a positive control, the scavenging effect of the extracts was lower. These results showed that extracts obtained from roasted coffee had ABTS radical scavenging activities, though the activities of the tested samples were lower than that of vitamin C.

Antioxidants can be referred to as reductants, which inactivate oxidants, and are involved in redox reactions in which the reaction species (oxidant) is reduced at the expense of the oxidation of the antioxidant (reductant). The FRAP assay measures the antioxidant effect of any substance in the reaction medium with reducing ability (19). Antioxidant potentials of various extracts obtained from roasted coffee were estimated by their ability to reduce TPTZ-Fe(III) complex to TPTZ-Fe(II) complex. In the present study, the trend for ferric ion reducing activities of extracts obtained from roasted coffee was shown in Fig. 1B. For roasted coffee, the absorbance clearly increased due to the formation of the Fe^{2+}-TPTZ complex with increasing concentration. FRAP of green bean, roasted coffees and the positive control decreased in the following order: vitamin C (2.30)> medium (2.03)> green bean (1.90)> city (1.47)> french (1.16) at the concentration of 1,000 μg/mL. Similar to the results obtained from the ABTS radical scavenging activity assay, extracts of medium roasted coffee showed relatively strong ferric ion-reducing activity. A correlation between mean values of the total phenolics and antioxidant activities of medium roasted coffee deserves detailed attention, as phenolics in roasted coffee were likely capable of reducing ferric ions (Fig. 1B). Some researchers have reported similar correlations between phenolics and antioxidant activity measured by various methods (20). Our results showed that a progressive decrease in antioxidant activity and polyphenol content was observed with longer roasting time; therefore, the light roasted

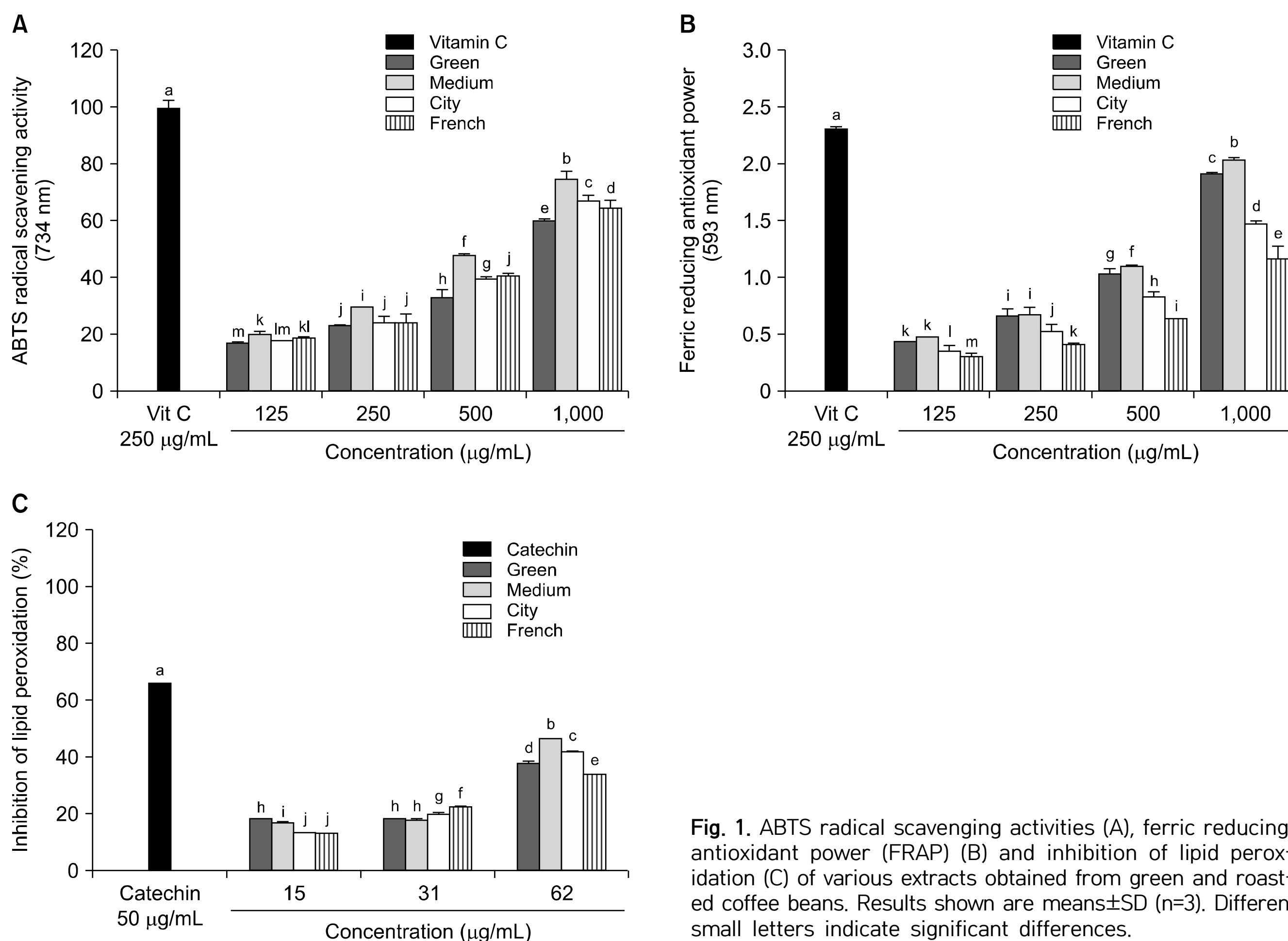

Fig. 1. ABTS radical scavenging activities (A), ferric reducing/antioxidant power (FRAP) (B) and inhibition of lipid peroxidation (C) of various extracts obtained from green and roasted coffee beans. Results shown are means±SD (n=3). Different small letters indicate significant differences.

coffee showed the highest antioxidant activity and dark roasted coffee showed the lowest antioxidant activity, similar to previous reports (21,22).

Inhibitory effect of green and roasted coffees on lipid peroxidation

Recently, the study of lipid peroxidation is becoming increasingly of interest because the formation of cytotoxic products such as malondialdehyde (MDA) and 4-hydroxynonenal can influence cellular apoptosis and several human diseases (22). Therefore, in this assay, antioxidant activities of roasted coffee extracts on both ferric ion and vitamin C-induced lipid peroxidation in mouse whole brain homogenates were also confirmed. Results shown in Fig. 1C revealed that medium coffee extracts have a significant higher activity in suppressing lipid peroxidation in mouse whole brain homogenates. Although medium coffee extracts presented lower than catechin as a positive control at all concentrations, also noteworthy was that the inhibitory effect on lipid peroxidation was not induced by a single compound but just 70% ethanol extracts. Therefore, medium coffee might be a potential and natural antioxidant supplement for healthy foods and functional food products.

Neuronal cell protection of green and roasted coffees on H_2O_2-induced neurotoxicity

H_2O_2 has been reported to induce apoptosis in cells of the central nervous system (5,6). In this study, extracts obtained from roasted coffee were selected to investigate the neuroprotective effects against H_2O_2-induced damage because of strong antioxidant activity. Protective effects on H_2O_2-induced neuronal cell damage were examined by the MTT assay. H_2O_2 caused a decrease in cell viability (37%), but pretreatment of PC12 cells with increasing concentrations of the medium coffee extracts inhibited oxidative stress-induced cytotoxicity (Fig. 2A). Neuronal cell protective effect of medium coffee extracts at 62 μg/mL on oxidative injury was similar to that of 200 μM vitamin C. This study demonstrated that PC12 cell cytotoxicity through oxidative stress was suppressed by pretreatment with extracts obtained from medium roasted coffee. MTT dye reduction assay is based on the catalytic activity of some metabolic enzymes in intact mitochondria (23). Mitochondria may be one of the most sensitive primary targets of oxidative injury in neuronal cells (24). These results suggest that PC12 cell protection by medium roasted coffee may partially be due to mitochondrial protective mechanisms.

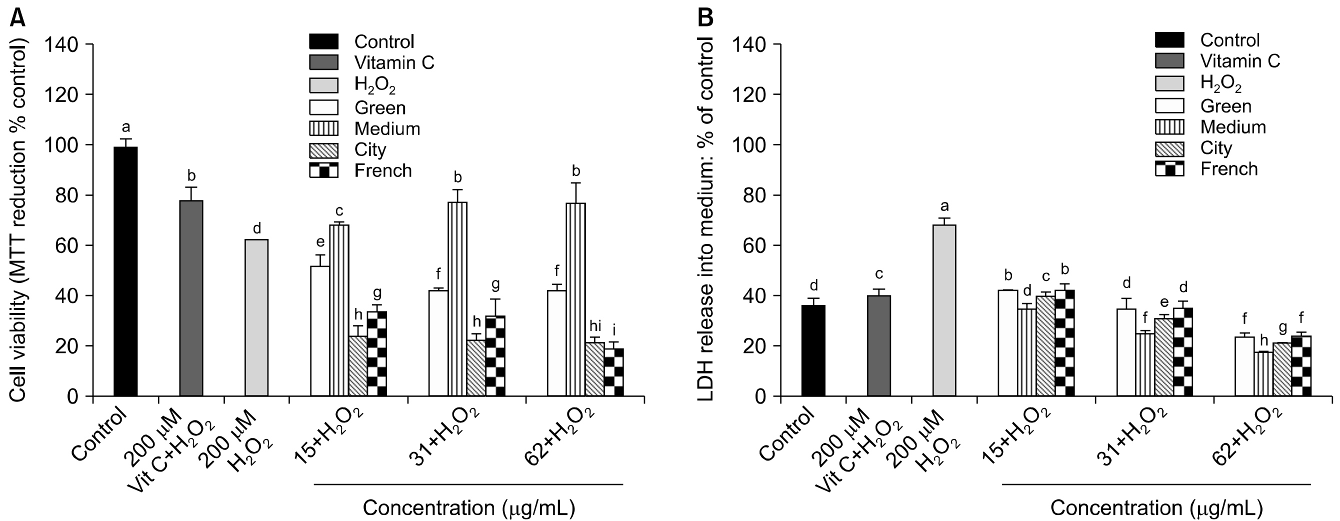

Fig. 2. Neuroprotective effects of various extracts obtained from green and roasted coffee beans on H_2O_2-induced cytotoxicity in PC12 cells. (A) Levels of cell viability were measured using the MTT assay. (B) LDH activity in culture supernatants was measured with a colorimetric LDH assay kit. Results shown are means±SD (n=3). Different small letters indicate significant differences.

Table 1. Total phenolics and phenolic composition of extracts from green and roasted coffee beans

		Green	Medium	City	French
Total phenolics (mg of GAE/g)		8.81±0.05[c]	9.77±0.03[b]	9.92±0.04[a]	7.76±0.01[d]
Phenolics content (mg/100 g)	5-O-caffeoylquinic acid	21.63±0.03[a]	14.7±0.04[b]	1.12±0.03[c]	0.66±0.05[d]
	Caffeic acid	2.67±0.03[c]	4.41±0.05[b]	0.53±0.05[d]	4.87±0.02[a]
	Syringic acid	0.87±0.02[b]	2.17±0.03[a]	0.84±0.03[c]	–[1)]

[1)]Not detected.
Results shown are means±SD (n=3). Different small letters indicate significant differences.

Protective effect of green and roasted coffees on H_2O_2-induced membrane damage

The neuronal membrane with polyunsaturated fatty acids is vulnerable to oxidative stress induced by ROS such as H_2O_2. Lipid peroxidation can alter the fluidity of the plasma membrane (18). LDH assay provided an estimate of the percentage of surviving PC12 cells. Green and roasted coffee extracts protected the integrity of the cellular membrane at all concentrations tested (Fig. 2B). Treatment with 200 μM H_2O_2 caused an increase in LDH released into the medium (65%). Compared with their effects in the corresponding H_2O_2 treatment group, green and all roasted coffee extracts dose-dependently decreased LDH released extracellularly. In addition, extracts of medium roasted coffee showed that the pattern of neuronal cell protection was similar to the MTT assay. The above results indicate that medium roasted coffee could protect PC12 cells against the membrane lesion induced by H_2O_2 (Fig. 2B). Therefore, these data suggest that PC12 cell protection by medium roasted coffee is partially due to the mitochondrial and cellular membrane protective mechanisms on H_2O_2-induced neurotoxicity.

Our results suggest that phenolics of roasted coffees might be inhibiting neuronal apoptosis, which is the ultimate consequence of these cellular dysfunctions. Especially, plentiful phenolics of medium-roasted coffees may also provide an added health benefit by reducing the risk of oxidative stress-induced neurodegenerative diseases.

Total phenolics and phenolic composition of green and roasted coffees

Phenolic compounds, such as flavonoids, phenolic acid and tannins, are considered to be major contributors to the antioxidant activity of natural plants. These antioxidants also possess diverse biological activities, including anti-inflammatory, anti-carcinogenic, and anti-neurodegenerative activities (25). Total phenolics of green and roasted coffee extracts were presented in Table 1. In addition, total phenolics of medium roasted coffee extracts were higher (9.77 mg GAE/g) than others. The extracts were subjected to further analysis by HPLC. The green and roasted coffee extracts contained various phenolic compounds. By comparing the retention time and UV spectra of these compounds with those of standards, 5-O-caffeoylquinic acid as the main phenolic was identified, followed by caffeic acid and syringic acid (Fig. 3). Furthermore, extracts from green coffee beans exhibited the highest 5-O-caffeoylquinic acid at 21.63 mg/100 g and in the following order medium (14.7 mg/100 g)> city (1.12 mg/100 g)> french (0.66 mg/100 g) (Table 1).

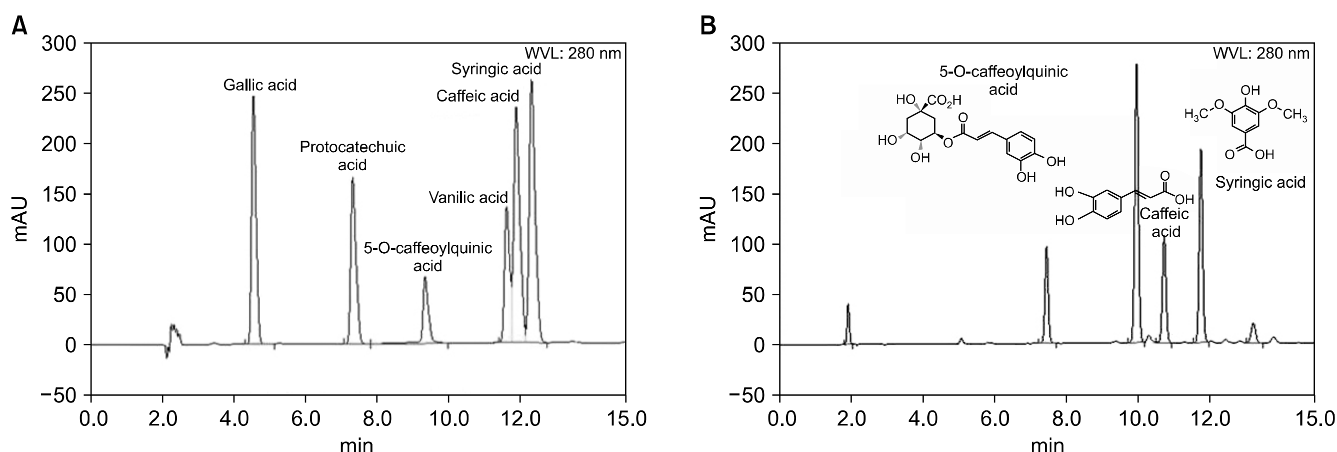

Fig. 3. HPLC chromatograms of various extracts obtained from medium roasted coffee beans. (A) Phenolics as compound standards, (B) Extracts obtained from medium roasted coffee beans. Retention times: gallic acid, 4.56 min; protocatechuic acid, 7.34 min; 5-O-caffeoylquinic acid, 9.37 min; vanillic acid, 11.65 min; caffeic acid, 11.92 min; syringic acid, 12.35 min.

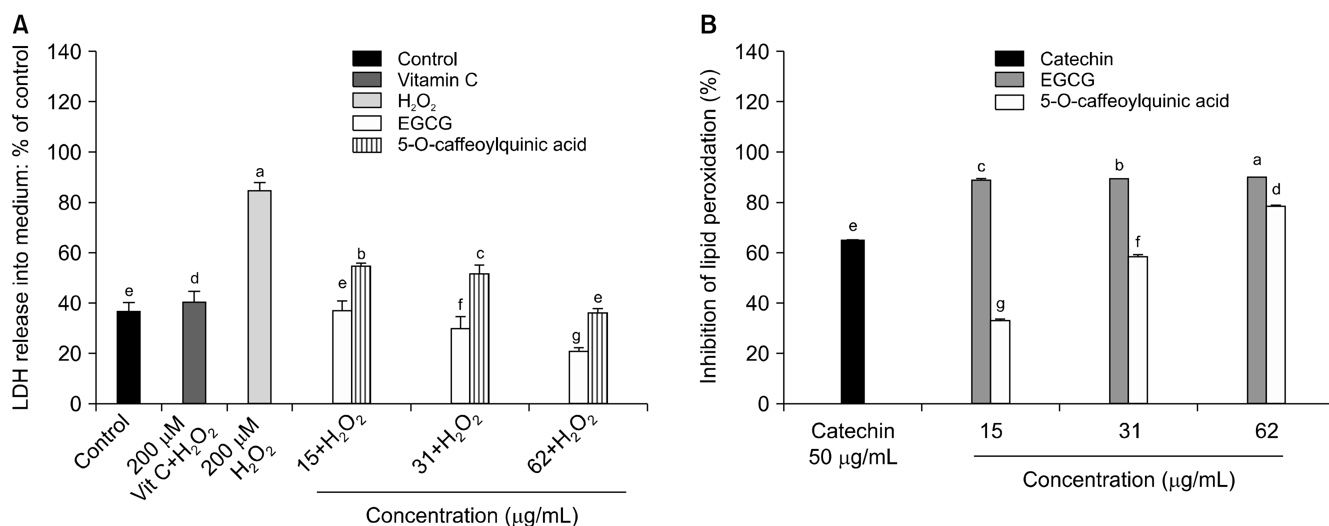

Fig. 4. Effect of 5-O-caffeoylquinic acid and epigallocatechin-3-gallate (EGCG) on lipid peroxidation. (A) Inhibitory effect on H_2O_2-induced membrane damage in PC12 cells. (B) Inhibitory effect on lipid peroxidation using mouse whole brain homogenates. Results shown are means±SD (n=3). Different small letters indicate significant differences.

In addition, when compared with other main phenolics as caffeic acid and syringic acid, the total sum of those in medium roasted coffee extracts was higher than another two coffee extracts. These results showed that many chemical component changes take place during roasting. Based on the results for the phenolic composition of extract from medium roasted coffee, we can conclude that these compounds (particularly 5-O-caffeoylquinic acid, caffeic acid and syringic acid) contribute to the protective effect on oxidative stress-induced neurotoxicity. The results obtained in this work are noteworthy, not only with respect to neuronal cell protections of roasted coffee extracts with phenolics but also with respect to compositional changes of phenolics due to roasting conditions.

Effect of 5-O-caffeoylquinic acid and epigallocatechin-3-gallate on lipid peroxidation

Today, green tea and coffee are the most widely consumed beverages by hundreds of millions of people around the world. Researches report that green tea has many health benefits because of physiological phenolics such as catechins. Catechins (flavan-3-ols) were the main phenolics in green tea, of which epigallocatechin-3-gallate (EGCG) represents approximately 59% of the total catechins (26). Therefore, we compared main phenolics of green tea and coffee on lipid peroxidation (Fig. 4). Compared with their effects on the corresponding H_2O_2 treatment group, EGCG and 5-O-caffeoylquinic acid dose-dependently decreased LDH released into medium (Fig. 4A). However, when compared with that of EGCG, membrane protective effect of 5-O-caffeoylquinic acid was lower. Similar to the results obtained from the LDH assay, EGCG and 5-O-caffeoylquinic acid both showed relatively strong inhibition of lipid peroxidation (Fig. 4B).

Antioxidant or oxidative stress studies of tea and coffee have been attracting more attention in recent years,

and the number of publications in this field increases every year. All of these publications include discussions of antioxidant capacity assays, comparisons of antioxidant activity of different phenolics and the chemical mechanism of scavenging ROS by phenolics (27,28). Because critical review papers on antioxidant activities of phenolics in green tea and coffee have been published, they are not a major point of discussion in this paper. Coffee and tea drinking are common worldwide, despite the internationally wide variety of drinking habits, e.g., type, frequency of intake, temperature, strength, etc. Although 5-O-caffeoylquinic acid showed lower antioxidant activity than that of EGCG at all concentration, this result was noteworthy because coffee has been one of the most popular beverages all over the world.

The physiological activities of roasted coffee extracts may be attributed to some phenolic compounds including 5-O-caffeoylquinic acid, caffeic acid and syringic acid. The content of 5-O-caffeoylquinic acid as a predominant phenolic compound was detected higher in medium roasted coffee extracts than others. Consequently, these data suggest that medium roasting condition in making tasty coffee from coffee beans may be more beneficial to human health, among them being the plentiful amounts of physiological phenolics including 5-O-caffeoylquinic acids.

ACKNOWLEDGMENTS

This work was supported by the National Research Foundation of Korea Grant funded by the Korean Government (KRF-2008-521-F00074, KRF-2011-0021664). This work was also supported by the Technology Development Program for Regional Industry of Ministry of Knowledge Economy, Republic of Korea (2009-70007068).

REFERENCES

1. Aruoma OI. 1996. Assessment of potential prooxidant and antioxidant actions. *J Am Oil Chem Soc* 73: 617-625.
2. Sawa T, Akaike T, Maeda H. 2000. Tyrosine nitration by peroxynitrite formed nitric oxide and superoxide generated by xanthine oxidase. *J Biol Chem* 275: 32467-32474.
3. Squadrito GL, Pryor WA. 1998. Oxidative chemistry of nitric oxide: the role of superoxide, peroxynitrite, and carbon dioxide. *Free Radic Biol Med* 25: 392-403.
4. Choi HR, Choi JS, Han YN, Bae SJ, Chung HY. 2002. Peroxynitrite scavenging activity of herb extracts. *Phytother Res* 16: 364-367.
5. Behl C, Lezoualch F, Trapp T, Widmann M, Skutella T, Holsboer F. 1997. Glucocorticoids enhance oxidative stress-induced cell death in hippocampal neurons *in vitro*. *Endocrinology* 138: 101-106.
6. Valencia A, Morán J. 2004. Reactive oxygen species induce different cell death mechanisms in cultured neurons. *Free Radic Biol Med* 36: 1112-1125.
7. Youdim KA, Joseph JA. 2001. A possible emerging role of phytochemicals in improving age-related neurological dysfunctions: a multiplicity of effects. *Free Radic Biol Med* 30: 583-594.
8. Rosa M, Alonso S, Francesca S, Fabiano R, Karoly H. 2009. Botanical and geographical characterization of green coffee (*Coffea arabica* and *Coffea canephora*): chemometric evaluation of phenolic and methylxanthine contents. *J Agric Food Chem* 57: 4224-4235.
9. Sun CL, Yuan JM, Koh WP, Yu MC. 2006. Green tea, black tea and breast cancer risk: a meta-analysis of epidemiological studies. *Carcinogenesis* 27: 1310-1315.
10. Kuriyama S, Shimazu T, Ohmori K, Kikuchi N, Nakaya N, Nishino Y, Tsubono Y, Tsuji I. 2006. Green tea consumption and mortality due to cardiovascular disease, cancer, and all causes in Japan: the Ohsaki study. *JAMA* 296: 1255-1265.
11. Iso H, Date C, Wakai K, Fukui M, Tamakoshi A. 2006. The relationship between green tea and total caffeine intake and risk for self-reported type 2 diabetes among Japanese adults. *Ann Intern Med* 144: 554-562.
12. Odegaard AO, Pereira MA, Koh WP, Arakawa K, Lee HP, Yu MC. 2008. Coffee, tea, and incident type 2 diabetes: the Singapore Chinese Health Study. *Am J Clin Nutr* 88: 979-985.
13. Clifford MN. 1985. The composition of green and roasted coffee beans. In *Coffee Botany, Biochemistry and Production of Beans and Beverage*. Clifford MN, Willson KC, eds. Chapman and Hall, London, UK. p 457.
14. Clifford MN. 1999. Chlorogenic acids and other cinnamates: nature, occurrence and dietary burden. *J Sci Food Agric* 79: 362-372.
15. Parliment TH. 2000. An overview of coffee roasting. In *Caffeinated Beverages: Health Benefits, Physiological Effects and Chemistry*. Parliment TH, Ho CT, Schieberle P, eds. ACS Symposium Series 754, American Chemical Society, Washington, DC, USA. p 188-201.
16. Jeong CH, Choi GN, Kim JH, Kwak JH, Kim DO, Kim YJ, Heo HJ. 2010. Antioxidant activities from the aerial parts of *Platycodon grandiflorum*. *Food Chem* 118: 278-282.
17. Chang ST, Wu JH, Wang SY, Kang PL, Yang NS, Shyur LF. 2001. Antioxidant activity of extracts from Acacia confuse bark and heartwood. *J Agric Food Chem* 49: 3420-3424.
18. Heo HJ, Choi SJ, Choi SG, Shin DH, Lee JM, Lee CY. 2008. Effect of banana, orange, and apple on oxidative stress-induced neurotoxicity in PC12 cells. *J Food Sci* 73: H28-H32.
19. Siddhuraju P, Manian S. 2007. The antioxidant activity and free radicalscavenging capacity of dietary phenol extracts from horse gram (*Macrotyloma uniflorum* (Lam.) Verdc.) seeds. *Food Chem* 105: 950-958.
20. Awika JM, Rooney LW, Wu X, Prior RL, Cisneros-Zevallos L. 2003. Screening methods to measure antioxidant activity of Sorghum (*Sorghum bicolor*) and Sorghum products. *J Agric Food Chem* 51: 6657-6662.
21. Duarte SMS, Abreu CMP, Menezes HC, Santos MH, Gouvêa CMC. 2005. Effect of processing and roasting on the antioxidant activity of coffee brews. *Ciênc Tecnol Aliment* 25: 387-393.
22. Sevanian A, Ursini F. 2000. Lipid peroxidation in membranes and lowdensity lipoproteins: similarities and differences. *Free Radic Biol Med* 29: 306-311.
23. Wallace DC. 1992. Mitochondrial genetics: a paradigm for aging and degenerative disease? *Science* 256: 628-32.
24. Prasad MR, Lovell MA, Yatin M, Dhillon H, Markesbery WR. 1998. Regional membrane phospholipid alteration in Alzheimer's disease. *Neurochem Res* 23: 81-88.
25. Chung KT, Wong TY, Wei CI, Huan YW, Lin Y. 1998. Tannins and human health: A review. *Crit Rev Food Sci Nutr*

38: 421-464.
26. McKay DL, Blumberg JB. 2002. The role of tea in human health: An update. *J Am Coll Nutr* 21: 1-13.
27. Kaur C, Kapoor HC. 2001. Antioxidants in fruits and vegetables the millennium's health. *Int J Food Sci Technol* 31: 703-725.
28. Chen J, Ho CT. 1997. Antioxidant activities of caffeic acid and its related hydroxycinnamic acid compounds. *J Agric Food Chem* 45: 2374-2378.