Food Sci. Biotechnol. Vol. 15, No. 5, pp. 728 ~ 734 (2006)

Food Science and Biotechnology

© The Korean Society of Food Science and Technology

Antioxidant Properties of Tannic Acid and its Inhibitory Effects on Paraquat-Induced Oxidative Stress in Mice

Je-Min Choi†, Jin Han[1†], Byoung-Seok Yoon, Jae-Hwan Chung[2], Dong-Bum Shin[3], Sang-Kyou Lee, Jae-Kwan Hwang*, and Ryung Ryang

Department of Biotechnology, College of Engineering, Yonsei University, Seoul 120-749, Korea
[1]1st R&D Center, Honda B/D, Samhwa F&F, Incheon 403-802, Korea
[2]Social Research and Data Analysis, World Research Corporation, Seoul 137-070, Korea
[3]Department of Food Science and Nutrition, Cheju National University, Jeju 690-756, Korea

Abstract The tannins represent a highly heterogeneous group of water-soluble plant polyphenols that may play an important role in antimutagenic and antioxidant properties. We investigated the antioxidant function of tannic acid in comparison to other phenolic compounds including catechin, chlorogenic acid, cinnamic acid, ellagic acid, and gallic acid for their ability to scavenge several stable radicals and reactive oxygen species (ROS) such as •DPPH$^+$, •ABTS$^+$, hydrogen peroxide, hydroxyl radical, and superoxide radical. The ability of tannic acid to decrease paraquat-induced lipid oxidation in mouse liver and lung through its antioxidant properties was also assessed. The results showed that almost all the tested compounds have stable radical scavenging activity except cinnamic acid. Tannic acid, gallic acid, and ellagic acid demonstrated remarkable ROS scavenging properties toward H_2O_2, •OH^-, •O_2^- and especially only tannic acid could inhibit paraquat-induced lipid peroxidation effectively in mouse liver and lung. Based on these results, it appears that increased number of galloyl and ortho-hydroxyl groups enhances the antioxidant activity of phenolic compounds and tannic acid is evaluated as the most effective antioxidant among all the tested compounds. These results suggest that the tannins, especially tannic acid, can be used as therapeutic agent for various diseases caused by ROS.

Keywords: tannin, tannic acid, antioxidant, ROS, paraquat, lipid peroxidation

Introduction

Plant tannins represent one of the most ubiquitous groups of natural polyphenols. Many pure tannins have significant biological and pharmacological activities, such as antimicrobial (1), antiviral (2), antioxidant (3), and antitumor activities (4-6). Tannins in wine have potent antioxidant activity against low-density lipoprotein (LDL) of which the oxidized form are a precursor of coronary heart disease (7). Tannic acid is a hydrolysable polyphenols which is found, along with other condensed tannins (8). It is most commonly found in the bark and fruits of many plants, in several beverages such as red wine and green tea, and has a structure consisting of many galloyl groups (9, 10) which may be associated with antioxidant activity (11).

Oxidative stress induced by oxygen radicals is believed to be a primary factor in various degenerative diseases as well as in the normal process of aging. Reactive oxygen species (ROS) are involved in the course of aging, cancer, cardiovascular disease, diabetes, neurodegenerative disease, and osteoporosis, etc. (12, 13). These diseases are closely related to daily food intake, and supplementation of plant-derived antioxidants would help to prevent these diseases. ROS such as hydrogen peroxide (H_2O_2), hydroxyl radical (•OH^-), and superoxide •O_2^- are highly reactive and can cause DNA mutation, protein degradation, and lipid peroxidation leading to loss of membrane integrity and cell death (14, 15). Injury caused by oxidative stress is one of the major damaging factors exposed to environmental stress such as paraquat (16-18). Paraquat is a potent pro-oxidant and reactive oxygens originated from it efficiently damage cellular components by oxidizing lipids, proteins, and nucleic acids (19). For this reason, paraquat has been widely used as a nonselective herbicide, but it is very toxic to humans and animals as well (20).

The objectives of this study are to determine the radical scavenging properties of tannic acid and other phenolic compounds including catechin, chlorogenic acid, cinnamic acid, ellagic acid, and gallic acid by *in vitro* analysis, and to evaluate the protective effect from paraquat-induced toxicity *in vivo*. This will lead to a greater understanding of the role of tannic acid in the prevention of ROS-induced damage in human diseases and to elucidate the relationship between its structure and antioxidant activity.

*Corresponding author: Tel: 82-2-2123-4097; Fax: 82-2-362-7265
E-mail: jkhwang@yonsei.ac.kr
Received May 6, 2006; accepted August 26, 2006
†Both authors contributed equally to the paper.

Materials and Methods

Materials All reagents were of analytic grade. Tannic acid, gallic acid, ellagic acid, chlorogenic acid, cinnamic acid, catechin, xanthine, xanthine oxidase (grade I), cytohrome C, horseradish peroxidase (Type IV), guaiacol, deoxyribose, 2-thiobarbituric acid, L-ascorbic acid, 1,1-diphenyl-2-picryl-hydrazyl (DPPH), 2,2'-azino-bis (3-ethylbenz-thiazoline-6-sulfonic acid): ABTS, paraquat, trichloroacetic acid (TCA), potassium persulfate, thiourea, disodium ethylene diamine tetraacetae (EDTA), and trolox were purchased from Sigma Chemical Company (St. Louis, MO, USA).

DPPH radical scavenging assay The antioxidant

activities of the aqueous extracts were measured on the basis of scavenging activity of the stable DPPH free radical following the method described by Braca *et al.* (21). Aqueous extract (0.1 mL) was added to 3 mL of a 0.004% methanol solution of DPPH. Absorbance at 517 nm was determined after 30 min, and percent inhibition was calculated as $[(Ao-A_i)/Ao] \times 100$ (Ao = absorbance without tannins, A_i = absorbance with tannins).

ABTS radical scavenging assay This assay is based on the scavenging of the relatively stable ABTS radical ($\bullet ABTS^+$) (22). ABTS radical was generated by the incubation of 7 mM ABTS with 2.5 mM potassium persulfate in the dark at room temperature for 12-16 hr, which was then diluted to 60 μM using a molar extinction coefficient of $\bullet ABTS^+$ at 734 nm (ε = 15/mM/cm). The $\bullet ABTS^+$ solution in water (2.5 mL) was mixed with test compounds, and 15 min later the absorbance of $\bullet ABTS^+$ was measured at 734 nm. Trolox Equivalent Antioxidant Capacity (TEAC) values were calculated from the slope of a plot of (Ao/A_i)-1 versus the compound concentration at (Ao/A_i)-1 = 1, where Ao is the absorbance in the absence of tested compound and A_i is the absorbance in the presence of tested compounds.

Hydrogen peroxide scavenging assay Hydrogen peroxide (H_2O_2) was measured by the formation of brown color in a reaction mixture containing 150 mM potassium phosphate buffer, 0.2%(v/v) guaiacol solution, and 10 μL of horseradish peroxidase (1,500 U/mL). The absorbance change was detected at 436 nm after reaction with guaiacol and 10 mM H_2O_2 for 30 min at room temperature (23).

Hydroxyl radical scavenging assay Hydroxyl radicals ($\bullet OH^-$) were generated by the Fenton system (ascorbic acid/$FeCl_3$-EDTA/H_2O_2) and the deoxyribose method was performed to measure hydroxyl radical scavenging activity (24). Reaction mixtures in a final volume of 1 mL contained, potassium phosphate buffer, pH 7.4 (10 mM), deoxyribose (2.8 mM), H_2O_2 (1.42 mM), $FeCl_3$-EDTA (20 and 100 μM), ascorbic acid (50 μM) and various test compounds. After incubation at 37°C for 1 hr, 1 mL of 2.8%(w/v) trichloroacetic acid (TCA) and 1 mL of 1%(w/v) thiobarbituric acid (TBA) were added, and the mixture was heated in a water bath at 100°C for 15 min. The absorbance of the resulting solution was measured at 532 nm.

Superoxide radical scavenging assay Catalysis of $\bullet O_2^-$ dismutation was measured using the cytochrome C reduction technique (25). Thus, catalysis was determined using xanthine oxidase (XOD) plus xanthine as the source of $\bullet O_2^-$ and ferricytochrome c as the indicating scavenger of $\bullet O_2^-$. The oxidation rate of cytochrome C was followed at 550 nm with a sensitive spectrophotometer. Catalysis was estimated in the presence of 0.1 mM EDTA in 50 mM potassium phosphate, pH 7.8, at 25°C.

Lipid peroxidation in the liver and lung of paraquat-treated mice Six-week-old male BALB/c (Charles River Technology, Chicago, IL, USA) mice were maintained under pathogen-free conditions. Mouse care and experimental procedures were performed under approval from the Animal Care Committee of Yonsei University. The mice were kept at 22°C and 20 to 50% humidity with a 12 hr light cycle. After 10 days of acclimation to the animal room, animals were divided into 5 groups of 10 mice each. Paraquat, gallic acid, ellagic acid, tannic acid, and diluent were injected intraperitoneally (i.p.). The control group of mice received phosphate buffered saline (PBS) only. The paraquat-injected mice received paraquat, in combination with tannins, in three injections of 10 mg/kg each with a 6 hr interval between the injections. Lipid peroxidation in the liver and lung of paraquat-treated mice was determined by measuring the amount of malondialdehyde (MDA) according to the method of a previous report (26) with some modifications. Freshly isolated liver and lung tissues (0.2 g) from control and tannic acid or phenolic compound-treated mice were homogenized in 5% TCA solution. The homogenates were centrifuged at 10,000×g for 15 min at 4 °C. Equal volumes of supernatant and 0.5% TBA in 20% TCA solution (freshly prepared) were added into a new tube and incubated at 95°C for 30 min in a water bath. The tubes were transferred into an ice bath for 5 min and then centrifuged at 8,000×g for 5 min. The absorbance of the supernatant was recorded at 532 nm. A solution of 0.5% TBA in 20% TCA was used as the blank. The MDA concentrations were expressed as nmole MDA per gram of tissue (nmol MDA/g tissue) which was determined using a coefficient of 155/mM/cm. Tissue levels of acid soluble thiols, mainly glutathione, were determined colourimetrically at 412 nm (27). Homogenates were precipitated with TCA and after centrifugation; supernatants were used for the estimation of glutathione (GSH) levels. The concentration of GSH was expressed as mol/g tissue.

Statistical analysis Data are presented as the means ± SD. All statistical tests were performed using SPSS for windows (version 13). Statistical analysis of group differences was examined using the non parametric Mann-Whitney-U test. Values of $p<0.05$ were considered to be significant.

Results and Discussion

Scavenging of the DPPH radical The DPPH radical is usually used as a substrate to evaluate the antioxidative action of antioxidants (28). DPPH is a stable free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The reduction in DPPH radical was determined by the decrease in its absorbance at 517 nm due to antioxidant activity. Figure 2 illustrates the scavenging effect of tannins on the DPPH radical. The scavenging effect increased with increasing concentration of tannins in the range of 1-100 μM. With cinnamic acid at concentrations of within this range, no significant difference in scavenging effect ($p>0.05$) was observed. For tannic acid at 10 μM, the scavenging effect on the DPPH radical was almost the same as 100 μM concentrations of the other compounds. These results show that phenolic compounds have remarkable hydrogen-donating ability with tannic acid being the most effective antioxidant among the other compounds. The range of hydrogen-donating ability observed for these compounds might be

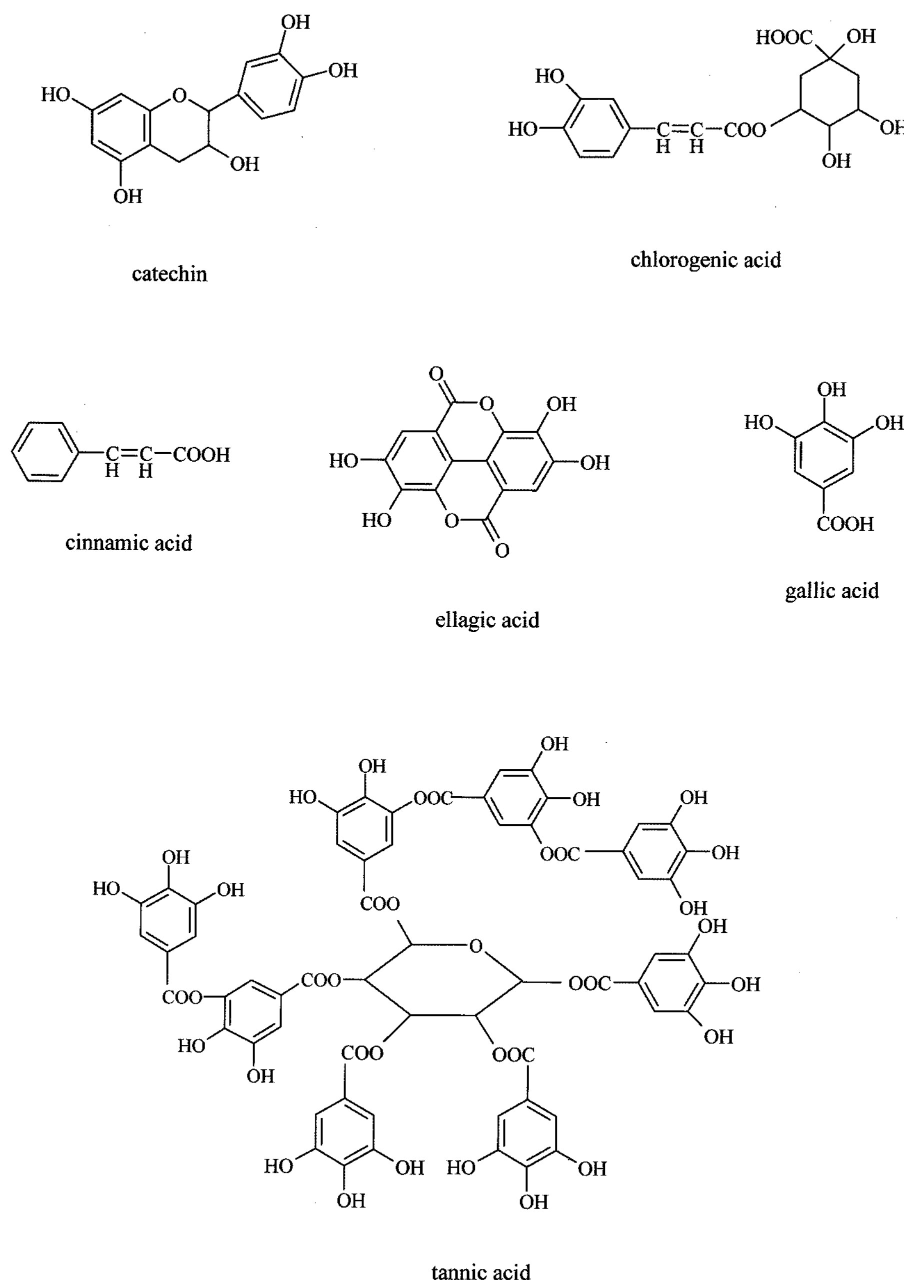

Fig. 1. Structures of tannic acid and phenolic compounds used in this study.

due to the number of hydroxyl groups in their structure (29).

Scavenging of the ABTS radical To confirm the antioxidant activity of tannic acid and other compounds, we used another stable radical, ABTS. Consistent with the results involving DPPH, chlorogenic acid, cinnamic acid, and ellagic acid showed relatively lower scavenging activity of the ABTS radical than tannic acid in the range 1-100 μM (Fig. 3). As shown in Fig. 1, cinnamic acid has only one carboxyl group, and galloyl group was not composed in the structure of chlorogenic acid, cinnamic acid, and ellagic acid however tannic acid has 5 galloyl groups. These structural characteristics of tannic acid might contribute to its antioxidant activity (29).

Hydrogen peroxide scavenging activity The scavenging effect of tannic acid and other phenolic compounds toward hydrogen peroxide is shown in Table 1. Data are presented in terms of the IC_{50} value, the concentrations which show a 50% scavenging effect against several ROS. The IC_{50} value of tannic acid (21.9±4.6 μM) is significantly lower than other phenolic compounds. Based on this result, tannic acid might play a role in signal transduction in apoptotic cells and protect cellular components from H_2O_2-induced toxicity (12, 13).

Hydroxyl radical scavenging activity The formation of $•OH^-$ was measured based on the 2-deoxyribose oxidative degradation and the production of MDA. All the tested phenolic compounds prevented hydroxyl radical-induced

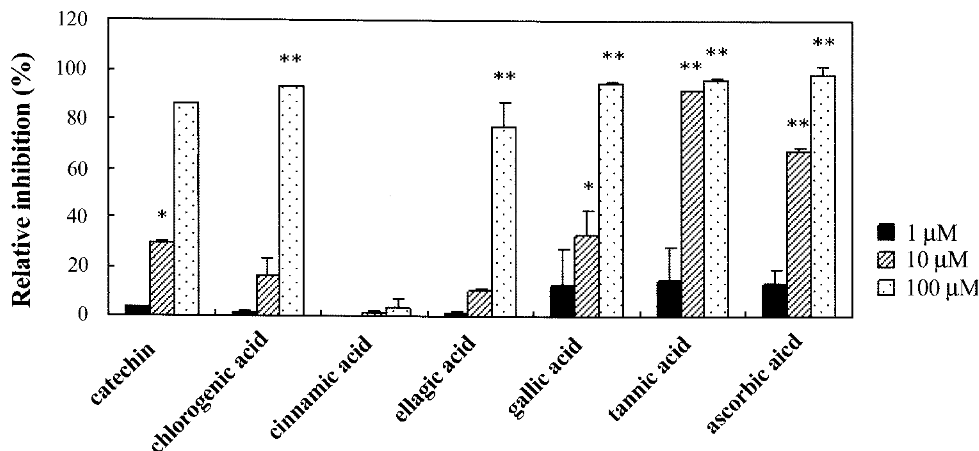

Fig. 2. DPPH radiacal scavenging activity of tannic acid and phenolic compounds. Several tannins (1-100 μM) were incubated with DPPH solution for 30 min at room temperature. Each value is the mean±SD (n=4). *$p<0.05$ and **$p<0.01$ relative to the PBS-treated group.

degradation of deoxyribose into MDA in a concentration dependent manner (data not shown). In Table 1, cinnamic acid had little effect on hydroxyl radical-induced color change. Catechin, ellagic acid, and gallic acid had a similar IC_{50} values which were higher than the IC_{50} value for tannic acid (89.4±18.1 μM). Many previous reports suggested that the hydroxyl group is essential factor for scavenging hydroxyl radicals, so the impressive hydroxyl radical scavenging activity of tannic acid might be due to its many -OH groups compared to other phenolic compounds (29).

Superoxide radical scavenging activity Superoxide radical is a highly toxic compound which is generated by numerous biological and photochemical reactions (15). Table 1 shows the results obtained in the $\bullet O_2^-$ scavenging assay. Tannic acid almost perfectly inhibited cytochrome C oxidation while gallic acid showed a little effect on superoxide radical at 1 mM (data not shown). The only IC_{50} value less than 1 mM was that of tannic acid, while the other compounds had IC_{50} values from 1-2.5 mM. Ellagic acid protected cytochrome C effectively against superoxide, and cinnamic acid showed radical scavenging activity only against the superoxide radical.

Inhibition of paraquat-induced lipid peroxidation in mouse liver and lung Liver and lung lipid peroxides, measured as MDA concentrations, from mice treated with paraquat were significantly increased compared to control mice (Fig. 4). Pre-treatment with tannic acid before paraquat injection significantly decreased the concentrations of MDA in the liver and lung compared to paraquat-treated mice and the control group. Paraquat injection produced a significant reduction of reduced glutathione in the liver and lung homogenate. Pre-treatment with tannic acid before paraquat injection significantly increased GSH content in the liver and lung compared to paraquat-treated mice (Fig. 5).

Results obtained in this study clearly demonstrate that the hydrogen donating capacity and ROS scavenging activity of tannic acid is more potent than other tested

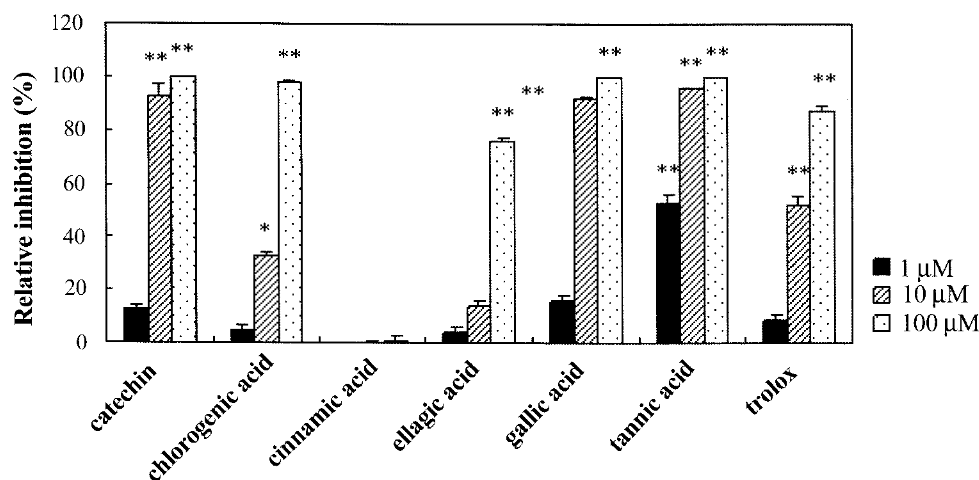

Fig. 3. ABTS radical scavenging activity of tannic acid and phenolic compounds. Several tannins (1-100 μM) were incubated with ABTS solution for 15 min at room temperature. Each value is the mean±SD (n=4). *$p<0.05$ and **$p<0.01$ relative to the PBS-treated group.

Table 1. Stable radical and ROS scavenging activities of tannic acid and phenolic compounds based on the IC_{50}[1] value (μM)

	•DPPH$^+$	•ABTS$^+$	H_2O_2	•OH$^-$	•O_2^-
Catechin	17.9±4.3	5.2±0.87	>1000 (606884.4±89436.5)	187.8±32.3	>1000 (1550.0±435.2)
Chlorogenic acid	51.4±12.2	12.7±1.2	703.3±98.2	397.1±11.2	>1000 (1550.0±101.4)
Cinnamic acid	n.s.[2]	n.s.	n.s.	>1000 (1852.5±435.2)	>1000 (1631.6±324.1)
Ellagic acid	64.2±11.4	63.2±9.8	>1000 (3395.1±1003.4)	149.1±15.7	>1000 (1148.2±768.4)
Gallic acid	12.1±2.3	5.0±0.3	>1000 (3870.6±84.5)	133.2±22.5	>1000 (2583.3±674.7)
Tannic acid	7.8±2.3	0.3±0.0	21.9±4.6	89.4±18.1	704.6±106.8

[1]IC_{50} is the concentration of the tested compounds which shows a 50% inhibitory effect.When 50% inhibition could not be reached at the highest concentration, the % of inhibition is given in parentheses. Data are presented as means±SD, n=4.
[2]n.s.; not significant.

phenolic compounds. Also, tannic acid effectively inhibited lipid peroxidation of mouse liver and lung tissue induced by paraquat, and remarkably increased GSH content in those organs.

Tannins are water-soluble polyphenols present in many foods and are recognized as antioxidants (10, 11). Tannins have been reported to have protective action against DNA damage (30, 31). Many tannin molecules have been shown to reduce the mutagenic activity of a number of mutagens and many carcinogens and/or mutagens produce oxygen free radicals which can damage cellular macromolecules (12, 30, 31). The anti-carcinogenic and antimutagenic

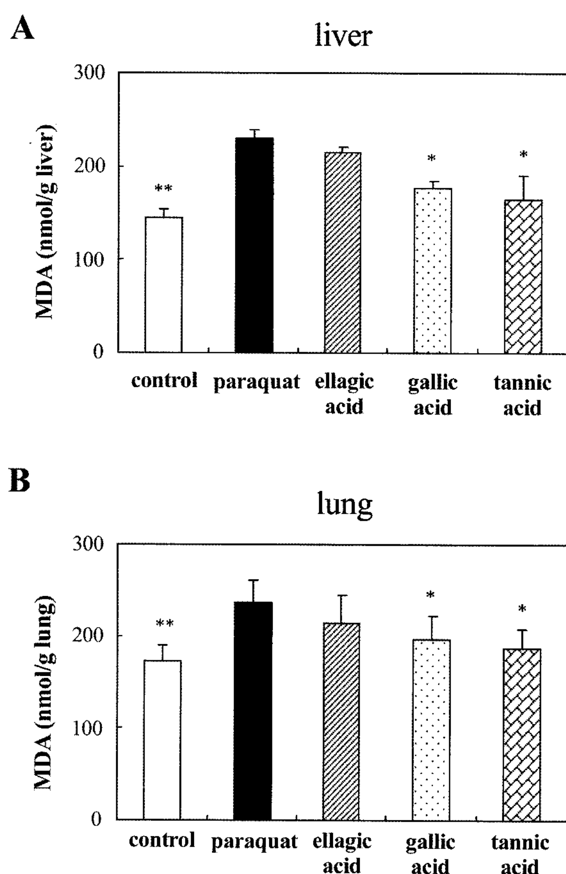

Fig. 4. Effect of tannic acid on paraquat-induced changes in lipid peroxide in mouse liver and lung. Each value is the mean ±SD (n=10). *p<0.05 and **p<0.01 relative to the paraquat-treated group.

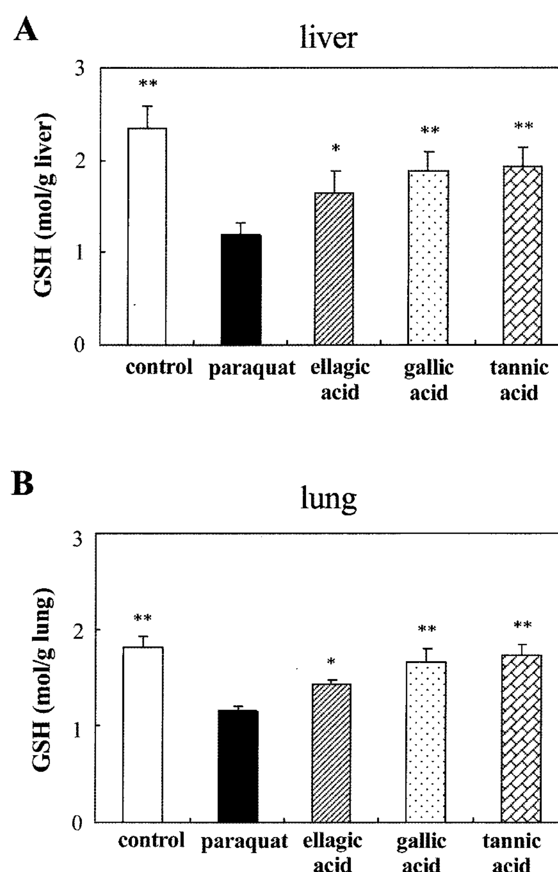

Fig. 5. Effect of tannic acid on paraquat-induced changes in total GSH contents in mouse liver and lung. Each value is the mean±SD (n=10). *p<0.05 and **p<0.01 relative to the paraquat-treated group.

potentials of tannins may be related to their anti-oxidative properties, which are important in protecting cells against oxidative damage (9). The generation of superoxide radicals was reported to be inhibited by tannins and related compounds (9). Tannic acid showed strong inhibitory activity toward the DPPH and ABTS stable radicals within a concentration range of 1-100 μM. Among the various tested phenolic compounds, there was an obvious correlation between the number of galloyl or hydroxyl groups and ROS scavenging activity. In the previous reports, galloyl groups can help the binding of Fe^{3+} and Cu^{2+} and the galloyl groups in tannins would appear to be associated with their inhibitory effects on Fenton reaction (H_2O_2+Fe^{2+} → •OH+OH+Fe^{3+})-induced hydroxyl radical formation (8, 11, 32). Except for cinnamic acid, all the tested phenolic compounds have many hydroxyl and galloyl groups, hence their significant hydroxyl radical scavenging activity in the present study. Interestingly, tannic acid demonstrated the most potent radical scavenging properties against stable radicals and ROS including •$DPPH^+$, •$ABTS^+$, H_2O_2, •OH^-, and •O_2^-. In its structure, tannic acid has many hydroxyl groups, suggesting that scavenging activity may be enhanced with increasing hydroxyl substitution. Based on these results, the ortho-hydroxyl group can be considered as the most important structural feature of tannins regarding their inhibition of free radicals. In previous reports, the ortho-hydroxyl group is a key structure in many antioxidant phenolic compounds such as flavonoids (11, 12).

Free radicals are known to play a crucial role in paraquat-induced lung toxicity (33, 34). It has been reported that this toxicity is related to redox cycling of an iron-paraquat complex, which in turn catalyzes the formation of ROS with the ultimate progression of lipid peroxidation (35, 36). In the current study, the elevation of lipid peroxide was taken as evidence for the involvement of oxidative stress in the development of paraquat-induced lung injury. Paraquat treatment was found to elevate the level of lipid peroxide in the liver and lung tissues, an observation which is in agreement with the previously reported data (36, 37). Paraquat has been demonstrated to be a highly toxic compound for humans and animals and many cases of acute poisoning and death have been reported over the past few decades (38, 39). The mechanisms of paraquat toxicity involve the generation of the superoxide anion which can lead to the formation of more toxic reactive oxygen species, such as hydrogen peroxide and hydroxyl radical. Also, lipid peroxidation, the oxidative degeneration of polyunsaturated fatty acids, has been suggested as a potential mechanism of paraquat toxicity in mammalian systems. On the other hand, the observed depletion of GSH could be a consequence of its consumption by paraquat-induced ROS generation. Glutathione (GSH) is the most abundant non-protein thiol in living organisms and it plays a crucial role in intracellular protection from toxic compounds, such as ROS and other free radicals (40). Several studies have demonstrated that GSH content in the lung, as well as in other tissues, is decreased in certain pathological conditions, such as hyperoxia, ischemia/reperfusion, and following the administration of oxidants such as paraquat. Therefore, GSH is important in conferring protection and preserving the integrity of the living organism (40, 41). In the present study, tannic acid significantly decreased MDA formation induced by paraquat and increased the GSH concentration in the liver and lung.

Tannins are essential components of the diet, which are concentrated in the peels of fruit and vegetables. The intake of these foods might have positive effects in many ROS-mediated diseases. There are several reports about the antioxidant activity of tannic acid, however this is the first report about the protective function of tannic acid against paraquat-induced oxidative stress in mice. In addition, we have demonstrated that tannic acid contains strong ROS scavenging activity than other phenolic compounds, and its antioxidant properties may be due to its hydroxyl group rich structure. Also, tannic acid can inhibit lipid peroxidation and increase GSH in concentration of mouse liver and lung tissue following paraquat-induced oxidative stress. Further studies are required to investigate the therapeutic effects of tannic acid on ROS-related diseases.

Acknowledgments

This work was supported by the Ministry of Commerce, Industry, and Energy for the Regional Innovation System program (Biofood Network Center) in 2004.

References

1. Burapadaja S, Bunchoo A. Antimicrobial activity of tannins from *Terminalia citrine*. Planta. Med. 61: 365-366 (1995)
2. Nakashima H, Murakami T, Yamamoto N, Sakagami H, Tanuma S, Hatano T, Yoshida T, Okuda T. Inhibition of human immunodeficiency viral replication by tannins and related compounds. Antivir. Res. 18: 91-103 (1992)
3. Satoh K, Sakagami H. Ascorbyl radical scavenging activity of polyphenols. Anticancer Res. 16: 2885-2890 (1996)
4. Gali HU, Perchellet EM, Klish DS, Johnson JM, Perchellet JP. Antitumor-promoting activities of hydrolyzable tannins in mouse skin. Carcinogenesis 13: 715-718 (1992)
5. Mukhtar H, Das M, Khan WA, Wang ZY, Bik DP, Bickers DR. Exceptional activity of tannic acid among naturally occurring plant phenols in protecting against 7,12-dimethylbenz(α)anthracene-, benzo (α)pyrene-, 3-methylcholanthrene-, and N-methyl-N-nitrosourea-induced skin tumorigenesis in mice. Cancer Res. 48: 2361-2365 (1988)
6. Miyamoto K, Nomura M, Murayama T, Furukawa T, Hatano T, Yoshida T, Koshiura R, Okuda T. Antitumor activities of ellagitannins against sarcoma-180 in mice. Biol. Pharm. Bull. 16: 379-387 (1993)
7. Teissedre PL, Frankel EN, Waterhouse AL, Peleg H, German JB. Inhibition of *in vitro* human LDL oxidation by phenolic antioxidants from grapes and wines. J. Sci. Food Agric. 70: 55–63 (1996)
8. Andrade RG, Dalvi LT, Silva JM Jr, Lopes GK, Alonso A, Hermes-Lima M. The antioxidant effect of tannic acid on the *in vitro* copper-mediated formation of free radicals. Arch. Biochem. Biophys. 437: 1-9 (2005)
9. Chung KT, Wong TY, Wei CI, Huang YW, Lin Y. Tannins and human health: a review. Crit. Rev. Food Sci. Nutr. 38: 421-464 (1998)
10. King A, Young G. Characteristics and occurrence of phenolic phytochemicals. J. Am. Diet. Assoc. 99: 213-218 (1999)
11. Okamura H, Mimura A, Yakou Y, Niwano M, Takahara Y. Antioxidant activity of tannins and flavonoids in *Eucalyptus rostrata*. Phytochemistry 33: 557-561 (1993)
12. Halliwel B, Gutteridge JMC. Free Radicals in Biology and Medicine, 3rd ed. Oxford Press, London, UK. p.11 (1998)
13. Enrique C, Kelvin JAD. Mitochondrial free radical generation, oxidative stress and aging. Free Radical Bio. Med. 29: 222-230

(2000)
14. Fruest EP, Norman MN. Interactions of herbicide with photosynthetic electron transport. Weed Sci. 39: 458-464 (1991)
15. Scandalios JG. Oxygen stress and superoxide dismutases. Plant Physiol. 101: 7-12 (1993)
16. Lannelli MA, Breusegem F, Montagu MV, Inze D, Massacci A. Tolerance to low temperature and paraquat-mediated oxidative stress in two maize genotypes. J. Exp. Bot. 50: 523-532 (1999)
17. Kwon SY, Jeong YT, Lee HS, Kim JS, Cho KY, Allen RD, Kwak SS. Enhanced tolerances of transgenic tobacco plants expressing both superoxide dismutase and ascorbate peroxidase in chloroplasts against methyl viologen-mediated oxidative stress. Plant Cell Environ. 25: 873–882 (2002)
18. Casano LM, Martin M, Zapata JM, Sabater B. Leaf age- and paraquat concentration-dependent effects on the levels of enzymes protecting against photooxidative stress. Plant Sci. 149: 13-22 (1999)
19. Moradas-Ferreira P, Costa V, Piper P, Mager W. The molecular defenses against reactive oxygen species in yeast. Mol. Microbiol. 19: 651-658 (1996)
20. Haly TJ. Review of the toxicity of paraquat. Clin. Toxicol. 14: 1-46 (1979)
21. Braca A, De Tommasi N, Di Bari L, Pizza C, Politi M, Morelli I. Antioxidant principles from *Bauhinia tarapotensis*. J. Nat. Prod. 64: 892-895 (2001)
22. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation declorization assay. Free Radical Bio. Med. 26: 1231-1237 (1999)
23. Aruoma OI, Halliwell B, Hoey BM, Butler J. The antioxidant action of taurine, hypotaurine, and therimetabolic precursors. Biochem. J. 256: 251-255 (1998)
24. Fernandes E, Costa D, Toste SA, Lima JL, Reis S. *In vitro* scavenging activity for reactive oxygen and nitrogen species by nonsteroidal anti-inflammatory indole, pyrrole, and oxazole derivative drugs. Free Radical Bio. Med. 37: 1895-1905 (2004)
25. McCord JM, Fridovich I. Superoxide dismutase. An enzymic function for erythrocuprein (hemocuprein). J. Biol. Chem. 244: 6049-6055 (1969)
26. Ohkawa H, Ohishi N, Yagi Y. Assay of lipid peroxides in animal tissue by thiobarbituric acid reaction. Anal. Biochem. 95: 351-358 (1979)
27. Ellman G. Tissue sulfhydryl groups. Arch. Biochem. Biophys. 82: 70-77 (1959)
28. Choi JM, Ryu HJ, Chung JH, Park JC, Hwang JK, Shin DB, Lee SK, Ryang R. Antioxidant property of genistein: Inhibitory effect on HOCl induced protein degradation, DNA cleavage, and cell death. Food Sci. Biotechnol. 14: 399-404 (2005)
29. Catherine AR, Nicholas JM, George P. Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radical Bio. Med. 20: 933-956 (1996)
30. Casalini C, Lodovici M, Briani C, Paganelli G, Remy S, Cheynier V, Dolara P. Effect of complex polyphenols and tannins from red wine (WCPTP) on chemically induced oxidative DNA damage in the rat. Eur. J. Nutri. 38: 190-195 (1999)
31. Giovannelli L, Testa G, De Filippo C, Cheynier V, Clifford MN, Dolara P. Effect of complex polyphenols and tannins from red wine on DNA oxidative damage of rat mucosa *in vivo*. Eur. J. Nutr. 39: 207-212 (2000)
32. Andrade RG, Ginani JS, Lopes GK, Dutra F, Alonso A, Hermes-Lima M. Tannic acid inhibits *in vitro* iron-dependent free radical formation. Biochimie 88: 1287-1296 (2006)
33. Tampo Y, Tsukamoto M, Yonaha M. Superoxide production from paraquat evoked by exogenous NADPH in pulmonary endothelial cells. Free Radical Bio. Med. 27: 588-595 (1999)
34. Minakata K, Suzuki O, Horio F, Saito S, Harada N. Increase in production of ascorbate radical in tissues of rat treated with paraquat. Free Radical Res. 33: 179-185 (2000)
35. Miguez MP, Soler F, Garcia-Rubio L. Accentuation of paraquat-induced toxicity by L-carnitine in mice. Biofactors 8: 73-78 (1998)
36. Tsukamoto M, Tampo Y, Sawada M, Yonaha M. Paraquat-induced membrane dysfunction in pulmonary microvascular endothelial cells. Pharmacol. Toxicol. 86: 102-109 (2000)
37. Venkatesan N. Pulmonary protective effects of curcumin against paraquat toxicity. Life Sci. 66: 21-28 (2000)
38. Autor AP. Biochemical mechanisms of paraquat toxicity. Life Sci. 14: 1309-1319 (1977)
39. Gram TE.. Chemically reactive intermediates and pulmonary xenobiotic toxicity. Pharmacol. Rev. 49: 297-341 (1997)
40. Rahman Q, Abidi P, Afaq F, Schiffmann D, Mossman BT, Kamp DW, Athar M. Glutathione redox system in oxidative lung injury. Crit. Rev. Toxicol. 29: 543-568 (1999)
41. Nam HY, Min SG, Shin HC, Kim HY, Fukushima M, Han KH, Park WJ, Choi KD, Lee CH. The protective effects of isoflavone extracted from soybean paste in free radical initiator treated rats. Food Sci. Biotechnol. 14: 586-592 (2005)