Antioxidant and Pro-Oxidant Effects of Various Tea Extracts

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The antioxidant and pro-oxidant effects of various tea extracts were investigated. The tea extracts, especially the green, pouchong, and oolong tea extracts, markedly stimulated the oxidation of deoxyribose in the presence of Fe^{3+} and H_2O_2. The rate of DNA degradation, induced by bleomycin-Fe^{3+}, was also accelerated by tea extracts (green tea, pouchong tea, and oolong tea) up to 65-fold at the dosage of 0.125 mg, and then decreased at higher dosages. Black tea exhibited greater stimulatory effects than the other three teas at higher dosage. The systems that contained only tea extracts and 2′-deoxyguanosine (2′-dG) do not induce the oxidation of 2′-dG to 8-hydroxy-2′-deoxyguanosine. Addition of ascorbic acid to the reaction system accelerated the oxidation of deoxyribose, DNA, and DNA bases, however, the oxidation was decreased by a high dosage of tea extracts. Tea extracts showed dual effects in the model system that was dependent on the ability of both reducing iron and scavenging oxy-radicals.

Keywords: *Tea extracts; pro-oxidant; deoxyribose; 2′-deoxyguanosine*

INTRODUCTION

The role of free radicals and active oxygen in the pathogenesis of certain human diseases, including cancer, aging, and atherosclerosis, is becoming increasingly recognized (Halliwell et al., 1992). Lipid peroxidation that involves a series of free radical mediated chain reaction processes is also associated with several types of biological damage. Therefore, much attention has been focused on the use of antioxidants, especially natural antioxidants, to inhibit lipid peroxidation or to protect the damage by free radicals.

The flavonoids are naturally occurring phenolic compounds in plants. The antioxidative effect of flavonoids has long been recognized. They have been reported to inhibit lipid peroxidation (Ratty and Das, 1988), to scavenge free radicals and active oxygen (Hanasaki et al., 1994), to chelate iron ions (Morel et al., 1994), and to inactivate lipoxygenase (Ratty et al., 1988). However, plant phenolics have sometimes been found to show pro-oxidant properties (Laughton et al., 1989). Several flavonoids have been shown to autoxidize and generate reactive oxygen species, such as hydrogen peroxide. They are also capable of reducing Fe^{3+} to Fe^{2+} and resulting in the formation of hydroxyl radicals by reacting Fe^{2+} with H_2O_2. Aruoma et al. (1992, 1993) reported that several phenolic antioxidants can accelerate oxidative damage of DNA, protein, and carbohydrates in vitro. Bonet et al. (1996) also indicated that vitamin C and flavonoids accelerate LDL oxidation induced by $CuCl_2$. Therefore, it is important to consider the pro-oxidant effects of phenolic antioxidant on biological molecules.

Tea has been used as a daily beverage and crude medicine in China for thousands of years. Polyphenols are the most abundant group of compounds in fresh tea leaves and are found in green and black tea beverages at 30−42% and 3−10% of the total dry matter, respectively (Graham, 1992). Pouchong and oolong tea are considered to be about $^1/_3$ and $^1/_2$ oxidized, respectively, as compared with black tea (Yamanishi, 1981). Hertog et al. (1993) reported that the average intake of all flavonoids was 23 mg/day, and the most important source of flavonoids was tea (48% of total intake). The pharmacological effects of tea are reviewed, including antioxidative activity (Matsuzaki and Hara, 1985), antimutagenic (Yen and Chen, 1994) and anti-cancer effects (Katiyar et al., 1992). In our previous work, the antioxidant activity of various tea extracts was compared. All tea extracts exhibited marked antioxidant activity, reducing power, and scavenging effects on active oxygen and free radicals (Yen and Chen, 1995). Although the antioxidant property of tea is recognized by some studies, further research on the role of the pro-oxidant ability of tea toward DNA and carbohydrates is required. The purpose of this work is to study the effects of various tea extracts (green tea, pouchong tea, oolong tea, and black tea) on the oxidative damage of deoxyribose, DNA, and DNA bases in vitro.

MATERIALS AND METHODS

Materials. Teas, including green tea, pouchong tea, oolong tea, and black tea, were purchased at a local market in Taichung, Taiwan. Deoxyribose, 2′-deoxyguanosine, bleomycin, and calf thymus DNA were purchased from Sigma Chemical Co. (St. Louis, MO). 8-Hydroxy-2′-deoxyguanosine was kindly supplied by Dr. T. Y. Liu (Dept. Med. Res. Veterans General Hospital, Taiwan). Thiobarbituric acid (TBA) and trichloracetic acid were purchased from E. Merck Co. (Darmstadt, Germany).

Preparation of Tea Extracts. Tea extracts were prepared according to the method described in our previous work (Yen and Chen, 1994). Briefly, each tea (20 g) was extracted with boiled water (400 mL) for 5 min, and the filtrate was freeze-dried.

Effects of Tea Extracts on Deoxyribose Damage. To test the ability of tea extracts to accelerate oxidative damage of deoxyribose, the Fenton reaction model system that contained $FeCl_3$-EDTA and H_2O_2 was used (Smith et al., 1992). The reaction mixture (3.5 mL), which contained tea extracts (0−12.5 mg), deoxyribose (3 mM), H_2O_2 (3 mM), KH_2PO_4−K_2HPO_4 buffer (20 mM, pH 7.4), $FeCl_3$ (50 μM), and EDTA (100 μM), was incubated at 37 °C for 1 h, with or without additional ascorbic acid (100 μM). The extent of deoxyribose degradation was measured by the TBA method. 1 mL of 1% TBA and 1 mL of 2.8% TCA were added to the mixture, which was then

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heated in a water-bath at 100 °C for 20 min. The absorbance of the resulting solution was measured spectrophotometrically at 532 nm. The control was without tea extracts and ascorbic acid. All analyses were run in three replicates and averaged.

Effects of Tea Extracts on Bleomycin-Dependent DNA Damage. The influence of tea extracts on the bleomycin-dependent DNA damage was determined according to the method of Aruoma et al. (1993). A solution (3.5 mL), which contained tea extracts (0−5 mg), calf thymus DNA (0.2 mg/mL), bleomycin (0.05 mg/mL), KH_2PO_4−K_2HPO_4 buffer (20 mM, pH 7.4), $FeCl_3$ (25 μM), and $MgCl_2$ (5 mM), was incubated at 37 °C for 1 h with or without additional ascorbic acid (240 μM). A portion (0.1 mL) of EDTA (100 mM) was added to the mixture, which was then measured by the TBA method as described above. The control was without tea extracts and ascorbic acid. All analyses were run in three replicates and averaged.

Effects of Tea Extracts on Oxidation of 2′-Deoxyguanosine. The effects of tea extracts on oxidation of 2′-deoxyguanosine (2′-dG) to 8-hydroxy-2′-deoxyguanosine (8-OH-2′-dG) were assayed, using a modified method of Kasai and Nishimura (1984). The reaction mixture (1.4 mL) contained tea extracts (0−7.5 mg), 2′-dG (0.5 mM), KH_2PO_4−K_2HPO_4 buffer (20 mM, pH 7.4), was initiated by the Fenton reaction model system [H_2O_2 (50 mM), $FeCl_3$ (1.3 mM), and EDTA (6.5 mM)] with or without additional ascorbic acid (15 mM). The entire mixture was incubated at 37 °C for 30 min, and incubation was terminated by placing the samples in an ice-bath, and then filtered through a 0.45 μm filter before use. The filtrate was analyzed by HPLC (Hitachi, Japan), using the LiChrosphere RP-18 column (150 mm × 4 mm, 5 μm) and UV detector (measured at 254 nm). The column was equilibrated with 50 mM KH_2PO_4 (pH 4.6)/methanol (93.5:6.5, v/v) at a flow rate of 0.7 mL/min. 2′-dG and 8-OH-2′-dG were identified by comparison of their retention times with those of known standards and determined by peak areas from the chromatograms. The control was without tea extracts and ascorbic acid. All analyses were run in three replicates and averaged.

Statistial Analysis. Data were analyzed using the Statistical Analysis System (SAS Institute Inc., 1985) software package. Significant differences between means were determined by Duncan's multiple-range tests.

RESULTS AND DISCUSSION

Effects of Tea Extracts on Deoxyribose Damage. The sugar deoxyribose whose exposure to hydroxyl radicals, generated by the Fenton reaction model system, degrades into fragments and generates a pink chromogen on heating with TBA at low pH (Halliwell et al., 1987):

$$Fe^{2+}\text{-EDTA} + H_2O_2 \rightarrow OH^- + {}^{\bullet}OH + Fe^{3+}\text{-EDTA}$$

$${}^{\bullet}OH + \text{deoxyribose} \rightarrow \text{fragments} \xrightarrow[\text{plus acid}]{\text{heat with TBA}} \text{chromogen}$$

This reaction is inhibitable by scavengers of the hydroxyl radical and hydrogen peroxide. However, the rate of deoxyribose degradation may be increased by involving a reducing agent in the reaction mixture and maintaining a supply of Fe^{2+}:

$$Fe^{3+}\text{-EDTA} + \text{reductant} \rightarrow Fe^{2+}\text{-EDTA} + \text{oxidant}$$

$$Fe^{2+}\text{-EDTA} + H_2O_2 \rightarrow Fe^{3+}\text{-EDTA} + {}^{\bullet}OH + OH^-$$

Therefore, this simple model system that contained deoxyribose, Fe^{3+}-EDTA and H_2O_2, could be used to assess the pro-oxidant properties of samples. The effects of various tea extracts on the oxidative damage

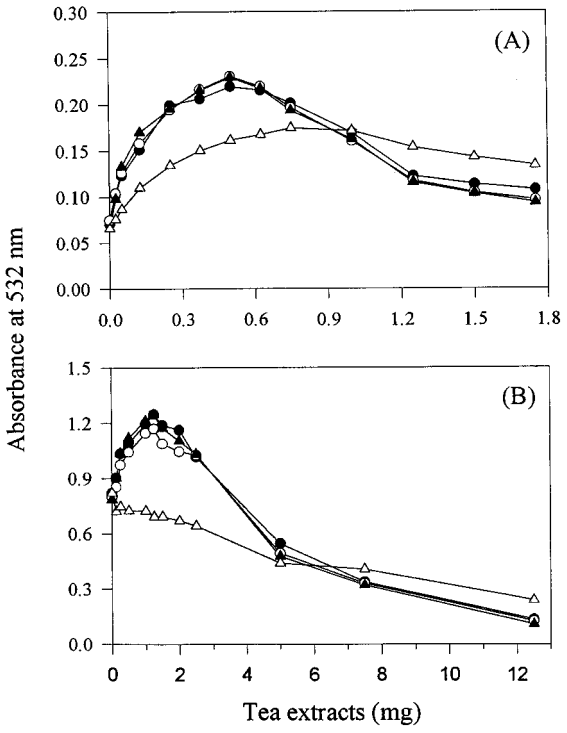

Figure 1. Effect of various tea extracts on the Fe^{3+}-H_2O_2-induced deoxyribose damage without additional ascorbic acid (A) or with additional ascorbic acid (B). (●) Green tea; (○) pouchong tea; (▲) oolong tea; (△) black tea.

of deoxyribose are shown in Figure 1. The stimulatory effects of green, pouchong, and oolong tea extracts increased with dosage (0.025−0.5 mg) up to a maximum and then decreased at the dosage 0.6−1.75 mg without additional ascorbic acid (Figure 1A). The oxidative damage of deoxyribose by 0.5 mg of green, pouchong, and oolong tea extracts was increased 3.1-, 3.1-, and 3.5-fold, respectively, when compared with the control. No significant difference ($P > 0.05$) was found for the pro-oxidant activity among green, pouchong, and oolong teas. The stimulatory effect of black tea extracts was weaker than the other three teas, and up to 2.6-fold of the control, at the dosage 0.75 mg. However, the reduction of the stimulatory effects of black tea extracts increased over 0.75 mg and was also weaker than the other teas.

Addition of ascorbic acid (100 μM) to the reaction system greatly increases the rate of ${}^{\bullet}OH$ generation (Figure 1B) by reducing Fe^{3+} to Fe^{2+} and maintaining the procession of the Fenton reaction. The oxidation damage of deoxyribose with additional ascorbic acid was 11.7-fold, as compared with the reaction system without additional ascorbic acid. When 0.125−12.5 mg of green, pouchong, or oolong tea extracts were added to reaction mixtures that contained additional ascorbic acid, they also accelerated the degradation of deoxyribose. However, the stimulation effects of these three tea extracts on the deoxribose oxidative damage were decreased at a dosage greater than 1.25 mg. Moreover, tea extracts at higher dosages exhibited the inhibitory effects on the deoxyribose oxidative damage, as induced by ascorbic acid. When black tea extracts were added to the system, the oxidation of deoxyribose was not in excess of that produced by ascorbic acid and was inhibited by increasing dosage. Tea extracts showed pro-oxidant effects on deoxyribose degradation at lower dosages without additional ascorbic acid; however, the stimulations of tea extracts were decreased at higher dosages. In the

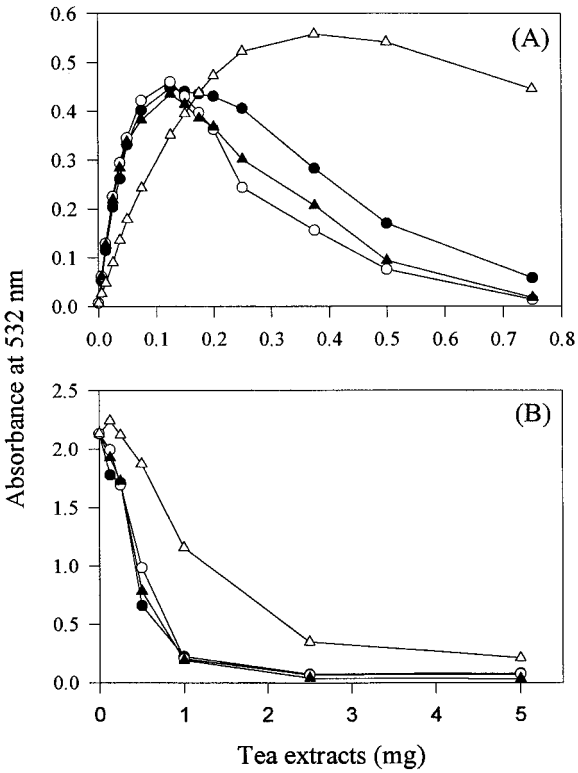

Figure 2. Effect of various tea extracts on bleomycin-Fe^{3+}-induced DNA damage without additional ascorbic acid (A) or with additional ascorbic acid (B). (●) Green tea; (○) pouchong tea; (▲) oolong tea; (△) black tea.

presence of additional ascorbic acid, tea extracts also exhibited antioxidative effects against deoxyribose degradation. Thus, tea extracts showed dual effects in vitro that were dependent on parameters such as dosage and transition metals. Aruoma et al. (1993) indicated that gallic acid was able to scavenge hypochlorous acid and superoxides and to inhibit the peroxidation of phospholipids. Nevertheless, gallic acid also accelerated the oxidative damage of deoxyribose. Several flavonoids, such as morin, quercetin, and myricetin, have been reported to accelerate the generation of the hydroxyl radical and to stimulate the degradation of deoxyribose in the presence of Fe^{3+} and H_2O_2 (Laughton et al., 1989). In our previous study, tea extracts showed marked reducing power and scavenging effects on active oxygen (Yen and Chen, 1995). Therefore, tea extracts might act as pro-oxidants or antioxidants, depending on their ability to reduce iron and scavenge oxy-radicals.

Effects of Tea Extracts on Bleomycin-Dependent DNA Damage. Bleomycin, an antitumor antibiotic, is able to bind to DNA and to cause single-strand breaks and degradation of the deoxyribose in the presence of O_2 and Fe^{2+}. The rate of DNA degradation induced by bleomycin-Fe^{3+} may be accelerated by containing a reducing agent in the reaction system. Nevertheless, some scavengers of active oxygen such as superoxide dismutase and catalase could decrease the damage of DNA (Halliwell and Gutteridge, 1989; Aruoma, 1991). Figure 2 shows the effects of various tea extracts on DNA damage induced by bleomycin-Fe^{3+}, with or without additional ascorbic acid. Green, pouchong, and oolong tea extracts accelerated the damage of DNA without additional ascorbic acid (Figure 2A). The stimulatory effects of these three tea extracts were 62.1−65.7-fold greater than that of the control at the dosage of 0.125 mg. However, the stimulatory effects of the three tea extracts were decreased by a dosage of tea extracts greater than 0.125 mg and were in decreasing order of green tea > oolong tea > pouchong tea. The DNA damage by addition of 0.75 mg of green, pouchong, and oolong tea extracts was 8.4-, 2.0-, and 2.6-fold, respectively, as compared with the control. Black tea extracts exhibited weaker stimulatory effects than the other three teas at lower dosage. However, the stimulatory effect increased with dosages of up to 79.7-fold of the control at an addition of 0.375 mg of tea extracts, and then mitigately decreased. The components of the black tea extracts were markedly different with the other three teas. Moreover, the reducing power of black tea extracts was also weaker than the other three teas (Yen and Chen, 1995). Thus, several compounds in black tea extracts may accelerate the bleomycin-Fe^{3+}-dependent DNA damage, and the results were independent of the ability of reducing power.

The rate of DNA damage was accelerated by addition of 240 μM ascorbic acid to reaction mixtures and was 305-fold as compared with the reaction mixtures without additional ascorbic acid. Contrasting with the deoxyribose assay, no stimulatory effects were found for any tea extracts except the black tea extracts at the dosage 0.125 mg. Moreover, all tea extracts exhibited the inhibitory effects on the DNA damage induced by bleomycin-Fe^{3+} and ascorbic acid. Over 96% of DNA damage was inhibited by green, pouchong, and oolong tea extracts at the dosage of 5 mg. Black tea exhibited the weakest inhibitory activity; it showed only 90% inhibitory effects at the same dosage. Gajewski et al. (1991) indicated that the mechanism of DNA damage in the bleomycin-Fe^{3+} system is not completely mediated by hydroxyl radical formation. Thus, the stimulatory or inhibitory effects of tea extracts on the bleomycin-Fe^{3+}-dependent DNA damage may be influenced by other factors besides the reducing power and the scavenger effects on active oxygen. Halliwell and Gutteridge (1989) reported that chelators such as EDTA could remove the remaining iron from the bleomycin−DNA complex to stop the reaction. The ability of flavonoids to form complexes with metal ions has also been demonstrated (Hudson and Lewis, 1983) and has been considered as a partial mechanism in the antioxidant action (Morel et al., 1994). Polyphenols are the most abundant group of compounds in tea extracts. The polyphenols in tea extracts can form a complex with iron ions to give a blue or purple color (Guo and Cheng, 1991). Thus, the inhibitory activity of tea extracts on bleomycin-Fe^{3+}-dependent DNA damage may be correlated with the chelating effects of iron ions.

Effects of Tea Extracts on Oxidation of 2′-Deoxyguanosine. The formation of 8-OH-2′-dG in the presence of Fe^{3+}/EDTA/H_2O_2 was analyzed by HPLC. The retention times for 2′-dG and 8-OH-2′-dG were 13.0 and 19.2 min, respectively (Figure 3a). Incubation of ascorbic acid and Fe^{3+}/EDTA/H_2O_2 led to a marked turnover of 2′-dG to 8-OH-2′-dG (Figure 3b,c). Tea extracts that exist alone in the reaction system do not cause the oxidation of 2′-dG (Figure 3d). However, tea extracts that coexist with Fe^{3+}/H_2O_2 accelerated the formation of 8-OH-2′-dG (Figure 3e,f). The effects of various tea extracts on the oxidation of 2′-dG are expressed in Figure 4. Tea extracts accelerated the formation of 8-OH-2′-dG with dosages (0.1−4.0 mg) up to a maximum without additional ascorbic acid and then gradually decreased. In comparisons with the control, the formation of 8-OH-2′-dG that were induced by green, pou-

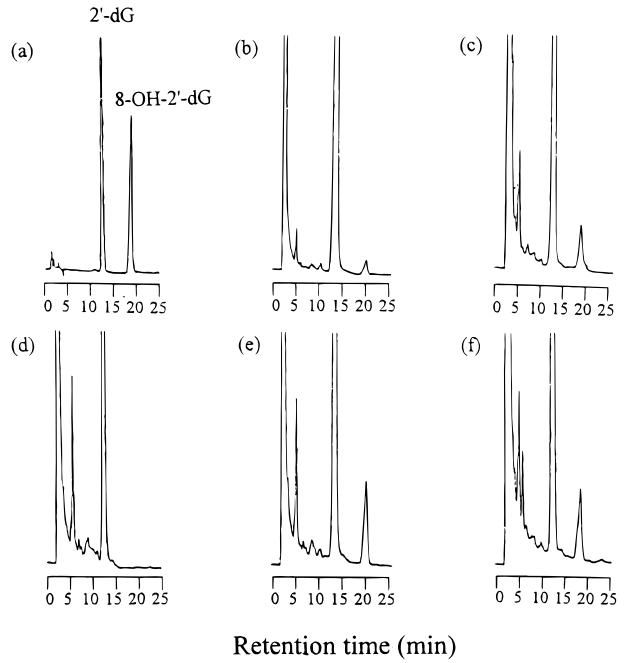

Figure 3. Detection of 2′-deoxyguanosine (2′-dG) and 8-hydroxy-2′-deoxyguanosine (8-OH-2′-dG) by HPLC in a reaction mixture of (a) 100 ng of 2′-dG and 100 ng 8-OH-2′-dG; (b) 0.5 mM 2′-dG, 1.3 mM $FeCl_3$, 50 mM H_2O_2, 6.5 mM EDTA, and 0.1 M phosphate buffer (pH 7.4) was shaken at 37 °C for 30 min; (c) as for b, but with 15 mM ascorbic acid; (d) 5 mg of oolong tea extracts and 0.5 mM 2′-dG were shaken at 37 °C for 30 min; (e) as for b but with 5 mg of oolong tea extracts; (f) as for b, but with 15 mM ascorbic acid and 5 mg of oolong tea extracts.

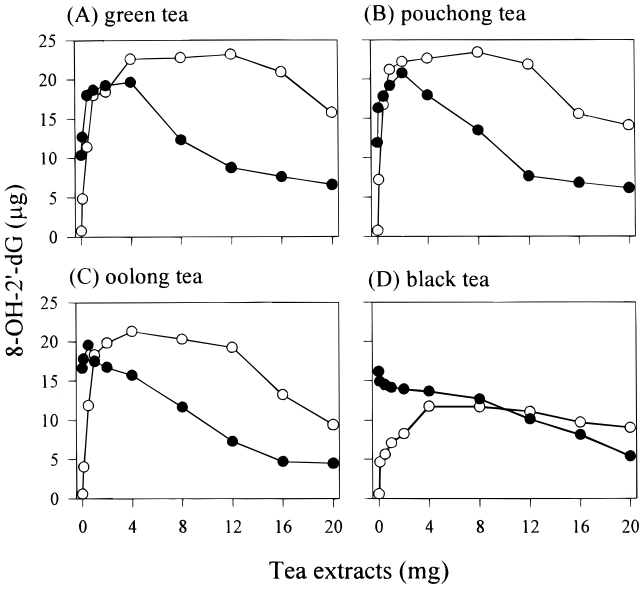

Figure 4. Effect of various tea extracts on the oxidation of 2′-dG to 8-OH-2′-dG induced by Fe^{3+}/H_2O_2 with additional ascorbic acid (●) or without additional ascorbic acid (○).

chong, and oolong tea extracts could be accelerated up to 30-fold greater. Black tea extracts exhibited weaker stimulatory effects than the other three teas. Addition of 15 mM ascorbic acid accelerated 15.7-fold the oxidation of 2′-dG; however, the stimulation would be decreased by a higher dosage of tea extracts. Stadler et al. (1994) indicated that incubation of caffeic acid led to the higher formation of 8-OH-2′-dG, resulting from the reductant ability of caffeic acid. Coffee also accelerated the oxidation of 2′-dG to 8-OH-2′-dG at lower additional dosages. However, the formation of 8-OH-2′-dG was decreased by increasing the dosage of coffee, which may be due to the interaction of the highly electrophilic hydroxyl radical with coffee constituents. In this system, tea extracts may act as pro-oxidants at lower dosage and may be dependent on the iron ions that exist in the model system. However, tea extracts also showed antioxidant activity at higher dosage by the ability of the scavenging effect of the hydroxyl radical.

CONCLUSION

The pro-oxidant property for some antioxidants has been increasingly studied in recent years. In these studies, the ability of ascorbic acid to act as a pro-oxidant is well documented (Laudicina and Marnett, 1990). In addition, many natural phenolic compounds are potent antioxidants (Bors et al., 1990). However, they can also act as pro-oxidants in some systems (Laughton et al., 1989). The pro-oxidant activity is a result of the ability to reduce metals, such as Fe^{3+}, to forms that react with O_2 or H_2O_2 to form initiators of oxidation. The oxidizing tissue injury in vivo that is induced by Fenton reaction is unclear and remains an important research issue. However, the Fenton reaction is the main biochemical source of hydroxyl radical. This reaction is dependent on the presence of non-protein-bound iron (Borg, 1993). Therefore, clarification of the role of redox-active metals and reducing agents as catalysts for the oxidation of molecules in vivo is interesting. The antioxidant activity of tea extracts is becoming increasingly recognized (Matsuzaki and Hara, 1985; Xie et al., 1993). In this study, our data also indicated that those tea extracts showed pro-oxidant effects at lower dosages. They may reduce Fe^{3+} and catalyze hydroxyl radical formation, which result in the oxidation damage of deoxyribose, DNA base, or DNA. Although these assays do not sufficiently represent the reaction in vivo, tea extracts that express antioxidant activity in lipid systems must be assessed for pro-oxidant properties in vivo.

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