Evaluation of the Antioxidant and Pro-oxidant Effects of Tea Catechin Oxypolymers

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Tea catechin oxypolymers (TCOP) were prepared by oxidizing tea catechin (TC, the content of EGCG was >85%) with H_2O_2. Their antioxidant and pro-oxidant effects were tested using a deoxyribose assay, a photoreduction of NBT assay, a lipoxygenase assay, a POV assay, and animal tests. The scavenging effects of TCOP to both the hydroxyl radical and superoxide radical were stronger than that of TC, and also they had no pro-oxidant effect; the rate constant for reactions of TC and TCOP for hydroxyl radical were 1.0×10^{10} and $(1.4-2.8) \times 10^{10}$ M^{-1} S^{-1}, respectively. TCOP can inhibit lipid peroxidation and lipoxygenase effectively, and it also can activate red cell SOD and reduce the MDA content in serum of mice very significantly. These results suggested that the antioxidant activity of TCOP was not less than or even more notable than that of TC.

Keywords: *Tea catechin oxypolymers; antioxidant; pro-oxidant*

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 (Haliwell 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 from damage by free radicals. Earlier studies have shown that plant phenolic compounds are effective antioxidants. Green tea and its polyphenol constituents were reported to possess antioxidant activity. They have been reported to inhibit lipid peroxidation (Ratty and Das, 1988; Xie et al., 1993), to scavenge free radicals and active oxygen (Hannasaki et al., 1994), and to inactivate lipoxygenase (Xie et al., 1993). However, plant phenolics have sometimes been found to show pro-oxidant properties (Laughton et al., 1989; Gow et al., 1997). 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+}, 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. Tea polyphenol (TP) was also reported to have pro-oxidant effects at lower dosages in the aqueous phase (Yen et al., 1997).

Oxidation was regarded to have a negative effect on the biological activities of tea catechins in the past, but some recent research has illustrated that oxyproducts formed from catechins may have more significant activities than catechins. Xie et al. (1993) reported that theaflavin monogallate B and theaflavin digallate can inhibit lipoxygenase more effectively than EGCG, ECG, and EGC. Hagerman et al. (1998) reported that high molecular weight plant polyphenols such as tannins were excellent biological antioxidants and that polyGG (polygalloyl glucose) has no pro-oxidant effect in the deoxyribose assay. Roeding-Penman et al. (1997) suggested that products formed from the catechins, for example, dimers, play important roles as antioxidants during the storage of foods containing added tea extracts. Black tea was also reported to have weaker stimulatory effects than green tea, oolong tea, and pouchong tea on bleomycin-dependent DNA damage at lower dosages (Gow et al., 1997). Therefore, polymeric phenols may be more effective antioxidants than simple phenols. Many investigations of the antioxidant and pro-oxidant effects of simple phenols have been done, but few have been done on the polymer of phenol.

This paper aimed to study the effect of oxidizing on the antioxidant activity of TC, and the antioxidant and pro-oxidant effects of tea catechins oxypolymers (TCOP) were evaluated.

MATERIALS AND METHODS

Materials. Nitro blue tetrazolium (NBT), thiobarbituric acid (TBA), and 2-deoxy-D-ribose (DR) were purchased from Sigma Chemical Co. (St. Louis, MO). SOD and MDA reagent boxes were purchased from Nanjin JianChen Biological Engineering Institute. Lipoxygenase was extracted from soybean and purified by our laboratory. All other chemicals used were of analytic purity. Lard oil was purchased at a local market in Wuhan, People's Republic of China.

Preparation of Crude Catechins. Green tea (100 g) was mixed with 500 mL of 80% ethanol and shaken at 30 °C for 24 h. After filtration, the solvent (ethanol) was removed with a rotatory evaporator under vacuum. The residues were dissolved in water and then extracted with chloroform and ethyl acetate three times (1:1, v/v). The ethyl acetate extracts was dried under vacuum by a rotatory voporator to get the crude catechins.

Purification of Crude Catechins. Three grams of dried crude catechins dissolved in 10 mL of 95% ethanol was applied onto a glass column (26 mm i.d. × 800 mm) packed with 100 g of Sephadex LH-20 (Pharmacia LKB). The column was connected to a Varian pump (Pharmacia LKB) equipped with an LKB 2138 UV detector at 280 nm. The mobile phase was 80% ethanol. Caffeine, ECG, EGC, and EGCG were eluted

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separately from the column. The last fraction (EGCG) was collected. After removal of ethanol, the purified catechins were obtained with the main constituents of EGCG (>85%), caffeine (2.7%), and ECG (~5%).

Preparation and Purification of Tea Catechins Oxypolymers. Ten percent of the above purified catechin (EGCG > 85%) with aquatic solution as substrate was mixed with 2.5% (v/v), oxidize (H_2O_2), and then reacted at 45 °C for 6 h with stirring at medium velocity. After ending the reaction, the mixture were freeze-dried to obtain TCOP. The main components of TCOP were oxypolymers (>80%), EGCG (7.1%), caffeine (2.6%), and ECG (0.7%).

TCOP was purified by Sephadex LH-20 as described above except that the mobile phase was 95% ethanol and 50% acetone; 95% ethanol was used to remove the residue caffeine, EGCG, and ECG at first, and then 50% acetone was used to obtain the main fractions of TCOP.

The molecular weight distribution of TCOP was obtained on a column of TSKG (4000H + 3000H) with THF as eluent. The result showed that the molecular weight ranged from 433 to 2000, which suggested that it may be a dimer, trimer, tetramer, or pentamer of EGCG. The detailed molecular weight of the main fraction of TCOP was determined by MS (Finnigan) after being separated by HPLC (HP). It turned out that TCOP used in these experiments was a mixture of polymers with molecular range from 906.4 to 1962.4. ^{1}H NMR and ^{13}C NMR (Botanry DRX500) confirmed that they were mainly dimers and trimers of EGCG and the main linkage was C_4-C_8.

The isolation, purification, and identification techniques applied to these compounds will be reported in other papers.

Methods. *Lipid Peroxidant Assay.* Pure lard (pork fat) without any additives was used as the substrate to evaluate the antioxidant activity of TCOP. Two milligrams of each antioxidant was dissolved in 1 mL of 95% ethanol and added to oils at different concentrations (0.005, 0.01, and 0.02%). The control sample contained 1 mL of 95% ethanol without any antioxidant. Oil systems were thoroughly homogenized and stored at 30 °C in a water bath for 25 days with occasional stirring with a glass rod. The POV was measured every 5 days according to AOAC (1990) methods.

Lipoxygenase Assay. Lipoxygenase activity was analyzed according to the method of Block et al. (1988). Linoleic acid (reagent grade) was used as the substrate in a 0.1 M, pH 8.5, Tris buffer at 25 °C. The absorbency at 234 nm was recorded as a function of time on the Shimadzu UV-265FW UV spctrophotometer. A sample containing all of the reagents except the enzyme solution was used as the blank control. Various concentrations of inhibitor were added to the enzyme, and the mixture was incubated at 25 °C for 5 min. The residual enzyme activity was then measured as described above.

Deoxyribose Assay To Assess Antioxidant and Pro-oxidant Action. The deoxyribose method for determining the rate of reaction of hydroxyl radical with antioxidant was performed as described by Halliwell et al. (1987). Reaction mixtures contained, in a final volume of 1.0 mL, the following reagents at the final concentrations stated: deoxyribose, 60 mM; KH_2PO_4-KOH buffer, pH 7.4, 20 mM; $FeCl_3$, 100 μM; EDTA, 100 μM; H_2O_2, 1 mM; and ascorbic acid, 100 μM. Solutions of $FeCl_3$ and ascorbate acid were made up immediately before use in deaerated water. After incubation at 37 °C for 1 h, the color was developed by adding 1 mL of 1% TBA (w/v) and 1 mL of 25% (v/v) HCl, which was then heated in a water bath at 100 °C for 15 min. The absorbance of the resulting solution was measured spectrophotometrically at 532 nm. The control was without antioxidants. This assay was performed without ascorbic or EDTA to assess pro-oxidant actions (Aruoma, 1991; Aruoma et al., 1987). All analyses were run in three replicates and averaged.

Assay of the Scavenging Capacity of Superoxide Radical. The assay was performed by using the method of photoreduction of NBT (Beauchamp et al., 1971) but with some modifications. Reaction mixtures in a final volume of 3.0 mL contained 4.6 $\times 10^{-3}$ M NBT, 1 $\times 10^{-2}$ M methionine, 3.3 $\times 10^{-6}$ M riboflavin, 0.05 M phosphate buffer, pH 7.8, and various concentrations of sample. The color was developed by illuminating the mixtures at 4000 Lx for 15 min and then measuring the absorbance at 560 nm.

Table 1. Effect of Different Concentrations of TC and TCOP on the POV of Lard Oil System

sample	concn (%, w/w)	POV (mmol/L·kg) at X days					
		$X=0$	$X=5$	$X=10$	$X=15$	$X=20$	$X=25$
control	0.00	2.0	7.45	8.89	10.24	19.25	27.98
TC	0.005	2.0	6.48	7.21	10.74	14.39	16.23
	0.01	2.0	6.26	6.82	8.75	11.03	15.34
	0.02	2.0	6.38	8.94	9.91	12.27	17.35
TCOP	0.005	2.0	7.51	8.60	9.39	11.86	14.25
	0.01	2.0	5.40	5.81	7.89	10.27	12.01
	0.02	2.0	7.75	7.80	10.48	13.01	15.93

Animals and Treatments. Female mice (old mice, 12 months old; young mice, 2 months old) were purchased from TongJi Medical University laboratory animal center. The mice were housed in stainless steel wire-bottomed cages and acclimatized under laboratory conditions (25−28 °C, relative humidity = 65%, 12 h light/dark cycle) for 1 week before study. At the end of this period, mice were weighed and randomly assigned to four groups. The weight of the old mice at the beginning of the study was 47.6 ± 3.9 g, and a group of young mice weighing 18.5 ± 1.4 g acted as young control. All mice were weighed weekly during the study. The young and old control groups were fed with NS 10 mL/kg of body weight (bw), and the old groups were fed TCOP of 100, 200, and 400 mg/kg of bw, respectively. After 30 days, the mice were then ether-anesthetized, and blood was collected for estimation of the red cell SOD activity and serum MDA content.

Assay of SOD Activity in Red Cells and Content of MDA in Serum. SOD activity and MDA content were determined according to the method of Ji (1991); 1.5 mL of ice-cold NS was added to 40 μL of mouse blood and centrifuged at 2000*g* for 30 min at 4 °C. The resulting supernatant was discarded, and 2 mL of ice-cold distillation water was added; after homogenization, 0.1 mL of ice-cold 95% ethanol was added, the mixture was shaken for 30 s, and then 0.1 mL of ice-cold chloroform was added; the mixture was then extracted in a vortex oscillator for 1 min and centrifuged at 4000*g* for 30 min. The upper layer was red cell extraction solution after dilution 5 times, 6−7 μL of it was used for SOD activity determination, and 100 μL serum was used for MDA measurement.

RESULTS AND DISCUSSION

Antioxidant Activity of TC and TCOP in Lard Oil System. Preliminary tests showed that at 30 °C the induction times of control, TC, TCOP groups were 12, 15, and 19 days, respectively, when the POV reached 9 mmol/L·kg; but at 50 °C, when the peroxidant valve (POV) arrived at 30 mmol/L·kg, the induction times were 7., 9.5, and 10 days. At lower temperature, the antioxidant activity of TCOP was much better than at higher temperature because TCOP was unstable when the temperature was >50 °C. TC used in this test mainly contain EGCG, and it was easy to degrade at higher temperature. TC and TCOP were tested at various concentrations in the lard oil system at 30 °C, but TCOP's synergism with vitamin C (V_c), citric acid, and cyclic dextrin (1:1, w/w) were carried out at 80 °C to save time.

The results showed that TCOP had strong antioxidant activity at the tested concentrations, and the antioxidant activity of TCOP was stronger than that of TC at 30 °C. Both TCOP and TC showed the best antioxidant activity at a concentration of 0.01%. Figure 1 showed that Vc, citric acid, and β-cyclic dextrin had synergism to TCOP, and the effect of Vc was the best in all tested materials. After the addition of Vc, the antioxidative activity of TCOP was 5 times higher than that of TCOP alone at 80 °C. The mechanism may be that Vc itself

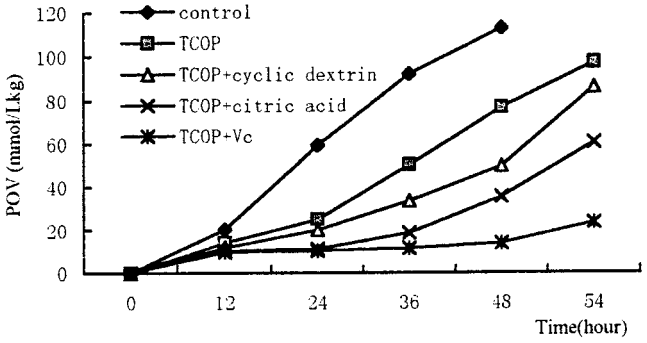

Figure 1. Synergism of cyclic dextrin, citric acid, and Vc to TCOP.

Table 2. Scavenging Effects of TC and TCOP on Superoxide Radical

	TC		TCOP	
concn (μg/mL)	ABS	scavenging ratio (%)	ABS	scavenging ratio (%)
0	0.469		0.469	
8.3	0.222	52.75	0.202	56.93
16.6	0.201	57.14	0.148	68.44
24.9	0.165	64.83	0.118	74.84
33.3	0.113	75.91	0.088	81.24
67.0	0.038	91.97	0.037	92.11
100	0.015	96.90	0.040	91.55
133	0.031	93.35	0.076	83.80
167	0.047	89.88	0.077	83.52

has a reduction ability to avoid lard oil oxidant, and it could spare TCOP and thus indirectly retard the oxidation of lard. Citric acid could chelate metal ions and enhance the hydrogen-donating ability of TCOP by forming a larger hydrogen donation with it.

Lipoxygenase Inhibitory Activity of TC and TCOP. The lipoxygenase inhibitory activities of TC and TCOP were measured, and the results were expressed as IC_{50} values (concentration at which 50% of enzyme activity is inhibited) in micrograms per milliliter. The IC_{50} values of TC and TCOP to lipoxygenase were 205 and 612 μg/mL, respectively.

Scavenging Capacity for Superoxide Radical ($O_2^{\bullet -}$). The biochemical mechanisms responsible for O_2 toxicity include lipid peroxidation and the generation of H_2O_2 together with the superoxide free radical, $O_2^{\bullet -}$ (Gutteridge et al., 1981). In the presence of traces of iron salts, $O_2^{\bullet -}$ and H_2O_2 can react together in vitro to form the hydroxyl radical $^{\bullet}OH$, which can attack and destroy almost all known biomolecules. It has been suggested that most, if not all, of the toxic effects of $O_2^{\bullet -}$ are due to the formation of $^{\bullet}OH$ from it in vivo. $O_2^{\bullet -}$ can be generated by photochemically reduced flavins, and it can reduce nitro blue tetrazolium to blue formazan, measured as a rise in absorbance at 560 nm (A), which can represent the content of $O_2^{\bullet -}$. After the addition of scavengers, the formation of formazan was inhibited, and the absorbance was A_1. The scavenging ratio can be calculated by the following formula: $S\% = [(A - A_1)/A] \times 100\%$.

Table 2 showed that both TC and TCOP have strong scavenging effects to the superoxide radical, and at the lower concentration range (8.3−33.3 μg/mL), the effect of TCOP was much stronger than that of TC. The scavenging effects of TCOP and TC increased with concentration (8.3−100 μg/mL) up to a maximum value and then decreased with increasing concentration.

Scavenging Effect of the Hydroxyl Radical. Antioxidants that have been shown to protect lipids against free radical chain reactions of peroxidation can be evaluated by their ability to prevent damage of carbohydrates using 2-deoxy-D-ribose as a substrate.

Table 3. Scavenging Effects of TC and TCOP on Hydroxyl Radical

	TC		TCOP	
concn (μg/mL)	ABS	scavenging ratio (%)	ABS	scavenging ratio (%)
0	0.517		0.517	
5	0.374	27.6	0.318	38.5
10	0.254	50.9	0.263	49.1
20	0.212	58.9	0.172	66.7
50	0.087	83.1	0.146	71.7
100	0.086	83.4	0.130	74.9
200	0.120	76.8	0.059	88.5
500	0.194	62.4	0.140	72.9
600	0.242	53.1	0.188	63.6
800	0.290	43.9	0.162	68.5

In the so-called deoxyribose assay (Halliwell et al., 1987) a mixture of Fe^{3+}-EDTA, hydrogen peroxide (H_2O_2), and ascorbic acid at pH 7.4 generates hydroxyl radical ($^{\bullet}OH$) (eqs 1 and 2) which can be detected by their ability to degrade the sugar deoxyribose into fragments that, on heating with thiobarbituric acid at low pH, generate a pink chromogen.

$$Fe^{3+}\text{-EDTA} + \text{ascorbate} \rightarrow Fe^{2+}\text{-EDTA} + \text{oxidized ascorbate} \quad (1)$$

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

In this system, addition of ascorbic acid greatly increases the rate of $^{\bullet}OH$ generation by reducing iron and maintaining a supply of Fe^{2+}. This ability to reduce Fe^{3+} and stimulate deoxyribose degradation has been adopted as one measure of pro-oxidant properties of actual and proposed food antioxidant additives (Laughton et al., 1989; Aruoma et al., 1992). The data in Table 3 show that both TC and TCOP could inhibit the formation of $^{\bullet}OH$ generated by the DR system effectively. Most redox active compounds react rapidly ($k = 10^9-10^{10}$ M^{-1} S^{-1}) with the hydroxyl radical ($^{\bullet}OH$), which is an extremely reactive free radical (Simic and Jovanovic, 1994). The DR method provides a convenient way to determine the rate constants for the reaction of water soluble compounds with $^{\bullet}OH$. Using that method, we obtained a second-order rate constant for TC (calculated by EGCG) of 1.0×10^{10} M^{-1} S^{-1}. TCOP was a potent inhibitor of color formation in the DR assay; the calculated second-order rate constant for the reaction of TCOP with $^{\bullet}OH$ was $(1.4-2.8) \times 10^{10}$ M^{-1} S^{-1}.

Compounds that act as pro-oxidants are thought to be detrimental because they may enhance oxidative damage. Some pro-oxidant compounds are able to redox cycle the metal ion required for $^{\bullet}OH$ generation and thus increase the radical concentration. We modified the DR method by omitting ascorbic acid (Aruoma, 1991) to evaluate the potential of TC and TCOP to behave as pro-oxidants. In this modification of the method, a pro-oxidant was substituted for ascorbic acid in the Fenton reaction and a decrease of color formation over the ascorbic acid-free controls was seen. We found that TC was a very effective substitute for ascorbic acid when the concentration was >500 μg/mL. This was consistent with the results of Shen et al. (1992), but TCOP was unable to substitute for ascorbic acid in the standard

Table 4. Absorbances Obtained in the DR Assay in the Presence and Absence of Ascorbic Acid (−AA) or EDTA (−EDTA)

condition	normal	−AA	−EDTA
no test substance	0.682	0.134	0.551
TC (200 μg/mL)		0.129	0.320
TC (500 μg/mL)		0.085	0.216
TC (1000 μg/mL)		0.438	0.285
TC (2000 μg/mL)		0.565	0.205
TCOP (200 μg/mL)		0.113	0.358
TCOP (500 μg/mL)		0.106	0.329
TCOP (1000 μg/mL)		0.136	0.150
TCOP (2000 μg/mL)		0.159	0.087

DR assay because TCOP, with a higher oxide potential than TC, is not a pro-oxidant (Table 4).

Some compounds inhibit color formation in the DR assay, not by reacting with •OH but by chelating iron-(III). To identify compounds that chelate metals, the DR assay is performed without EDTA (Aruoma et al., 1987). Inhibition of iron-dependent DR degradation in the absence of EDTA depends not only on the ability of a scavenger to react with •OH but also on its ability to form a complex with iron ions (Halliwell et al., 1987). Table 4 also shows that both TCOP and TC had no pro-oxidant effect in the absence of EDTA, and the mechanism included not only a scavenging effect but also an iron chelating effect, because the reduction of absorbance was not in a linear relationship with the increase in concentration. However, the iron chelating effect of TCOP was more notable than that of TC at the higher concentration.

Effects of TCOP on the Body Weight, Red Cell SOD Activity, and Serum MDA Content. The body weights of mice in each group are given in Table 5. It is suggested that TCOP had no negative effect on mice. The weights of the young mice group increased quickly because they were in a vigorous growing period.

It was found that, compared with the old mice control, the activities of SOD in all tested groups were significantly increased ($P < 0.01$), the concentration of MDA decreased significantly ($P < 0.01$), and the activities of SOD of the medium- and high-dosage groups had no significant difference compared with the young control group. The MDA content was even lower than that of the young control group (but not significantly different). It seemed that TCOP could delay aging, and its excellent antioxidant activity maybe one mechanism of the oxidized catechins' and polymeric catechins' antimutagenicity.

As is well-known, the oxidation of lipid is a typical radical chain reaction. Oxygen can abstract α-hydrogen from unsaturated fatty acid and initiate a radical chain reaction. TCOP was formed by the oxidation and polymerization of EGCG; TCOP still contains many unoxidized phenolic hydroxyls, and perhaps it contained more phenolic hydroxyls than monomeric catechins because of polymerization. It can supply hydrogen to combine with lipid radical, and itself formed a stable radical to terminate the radical chain reaction of lipid.

It is thought that the lipoxygenase pathways of arachidonic acid metabolism produce reactive oxygen species and these reactive forms of oxygen and other arachidonic acid metabolites play a role in inflammation and tumor promotion (Huang et al., 1991). This study also suggested that both TC and TCOP can inhibit lipoxygenase, but the activity of TCOP was weaker than that of TC. Because the IC_{50} value can only reflect one certain inhibitory concentration, and many compounds did not act dose-dependently, perhaps some other evaluation should be adopted at the same time.

Reactive oxygen species might be important causative agents for a number of human diseases, including cancer, atherosclerosis, and aging. Tea polyphenols such as EGCG, EGC, and ECG have been proved to be able to react with peroxy radical and thus terminate lipid peroxidation chain reactions (Katiyar et al., 1994). To avoid oxidation induced by oxygen radicals, one of the most efficient means is to scavenge or inhibit reactive oxygen species. The scavenging ability of TCOP for two of the most reactive oxygen species, hydroxyl radicals and superoxide anion radicals, were investigated. The ability of TCOP to quench free radicals is more notable. TCOP are polymers oxidized from EGCG, and their high molecular weight and the proximity of many aromatic rings and hydroxyl groups are more important for free radical scavenging, which may have some similarity with tannins.

An important mechanism of human aging is lipid peroxidation and formation of excess radicals. Superoxide dismutase (SOD) is the enzyme responsible for degradation of the toxic superoxides. Although superoxides have limited chemical reactivity, they have the ability to generate more dangerous species, such as highly reactive hydroxyl radicals or the protonated form of superoxides (HO_2•) (Halliwell and Chirico, 1993). Decomposition of excess superoxides by SOD is an important physiological antioxidant defense mechanism in aerobic organisms. MDA was the product of lipid oxidation, and the content of MDA in an organism can reflect the degree of damage to the body. Free radicals can attack or destroy the SOD of a body. The SOD activity of a body reduced with age, which is shown by Table 6; the SOD activity of the old mice control group was below that of the young mice control by 18.7%. TCOP can spare SOD or prevent SOD damage or oxidation by scavenging free radicals very effectively and so increase the activity of SOD and reduce the content of MDA. It was also possible that TCOP could adjust the synthesizing of SOD in the body.

Many previous studies have focused on the antioxidant and antimutagenic effects of TC. Furthermore, the mechanisms of tea contributing to the anticarcinogenic and antimutagenic effects may involve antioxidant activity (Ho et al., 1992; Yen et al., 1995). However, few studies on polymers of TC were carried out. Hagerman (1998) proved that high molecular weight plant polyphenolics (tannins) may be more potent antioxidants than

Table 5. Changes of Body Weight of Mice ($\bar{x} \pm$ SD)

group	dosage (mg/kg of bw)	weeks 0	1	2	3	4
old control	0	47.3 ± 4.4	47.2 ± 3.8	47.5 ± 5.3	47.4 ± 4.6	46. ± 5.4
TCOP	100	47.9 ± 4.1	48.0 ± 3.8	47.5 ± 3.9	47.0 ± 3.7	46.4 ± 4.1
TCOP	200	47.4 ± 3.1	47.1 ± 3.2	47.0 ± 2.8	46.1 ± 3.1	45.8 ± 2.8
TCOP	400	47.7 ± 3.9	47.5 ± 3.6	47.1 ± 3.8	46.5 ± 4.1	46.0 ± 3.5
young control	0	18.5 ± 1.4	24.9 ± 1.6	29.5 ± 1.6	30.7 ± 1.8	31.5 ± 1.7

Table 6. Effect of Tea Polyphenol Oxyproducts on Antioxidative System of Mice

group	dosage (mg/kg of bw)	no.	SOD activity of red cells (Nu/mL), $\bar{x} \pm$ SD	content of MDA in serum (nmol/mL), $\bar{x} \pm$ SD
old control	0	10	20074.7 ± 1525.3	12.26 ± 2.55
TCOP	100	10	22027.7 ± 1247.9[a]	8.66 ± 1.79[a]
TCOP	200	10	23802.3 ± 1353.1[a,b]	7.67 ± 1.87[a,b]
TCOP	400	10	24216.0 ± 1445.0[a,b]	7.28 ± 1.54[a,b]
young control	0	10	24696.7 ± 1585.7[a]	8.37 ± 1.72[a]

[a] $P < 0.01$ compared with the old mice control. [b] $P > 0.05$ compared with the young mice control.

simple monomeric phenolics. The partial structure analysis of the TCOP we used here showed that they were mainly dimers to trimers of EGCG. Our study confirmed that slight oxidation (polymeric degree < 5) had no negative effect on the antioxidant activity of TC. Furthermore, polymers formed from catechins have more notable antioxidant activity, which also proved the suggestion of Roeding-Penman et al. (1997).

The antioxidant activity of food additives is usually evaluated only in lipid systems, but evidence exists that some small phenolics are pro-oxidants in nonlipid systems (Laughton et al., 1989; Aruoma et al., 1990). It is necessary to evaluate an antioxidant in both lipid and nonlipid systems. Our data suggested that TCOP was an excellent antioxidant in all lipid and nonlipid systems and enzyme and nonenzyme systems and had no pro-oxidant activity. The mechanism of the antioxidant of TCOP included quenching or scavenging of free radicals directly, chelating metal ions, activating antioxidant enzyme, and inactivating oxidant enzyme. The fine structures of TCOP constituents and the relationships between the structure and the antioxidative mechanism in living organisms need to be thoroughly investigated.

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