

Antioxidative Activities of Low-Caffeine Extract Prepared from Green Tea Leaves

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Abstract

The antioxidative activities of low-caffeine tea extract (LCTE) prepared from green tea leaves were compared with those of green tea extract (GTE). The yields of GTE and LCTE from tea leaves were 17.3% (w/w) and 10.2% (w/w), respectively. The catechin and caffeine contents of LCTE were *ca.* 43% and *ca.* 8% those of GTE, respectively. β -Carotene and α -tocopherol were detected only in LCTE at levels of *ca.* 0.3% (w/w). LCTE showed the same levels of iron-chelating and radical scavenging activities as GTE *in vitro*. To study antioxidative activities of LCTE *in vivo*, male ddY mice were given the following different diets for 6 weeks: normal diet, or that supplemented with 1.0% GTE, with 0.5% LCTE, or with 1.0% LCTE. Diets supplemented with 1.0% GTE, 0.5% LCTE, and 1.0% LCTE tended to lower lipid peroxide contents in mouse plasma in comparison to mice fed the normal diet. In the mouse liver, lipid peroxide contents in 1.0% GTE, 0.5% LCTE, and 1.0% LCTE groups were significantly decreased compared to those of the normal group. Retinol and α -tocopherol contents in the plasma of mice fed diets supplemented with 0.5% LCTE and 1.0% LCTE were significantly higher than those of mice fed the normal diet. These results suggest that supplementation of the diet with LCTE could elevate resistance to oxidative stress *in vivo*.

Keywords: Green tea, Low-caffeine extract, Antioxidant, Mouse

1. Introduction

Antioxidative activity of green tea leaves (*Camellia sinensis* L.) has been reported in some *in vitro* [1] and *in vivo* [2,3] studies. Green tea leaves contain several hydrophilic and hydrophobic antioxidants. Ascorbic acid [4,5] and four major catechins [6,7], (-)-epicatechin, (-)-epicatechin-3-*O*-gallate, (-)-epigallocatechin, and (-)-epigallocatechin-3-*O*-gallate, are the main hydrophilic antioxidants in tea leaves. Especially, the catechin contents of green tea

leaves are up to 15% dry weight [8]. These hydrophilic components would act as available antioxidants in the human body when ingested as a hot water infusion of tea. However, it was reported that *ca.* 30% of water-soluble materials could remain in the waste of tea leaves after a single extraction process with hot water [9]. On the other hand, green tea leaves also include considerable amounts of hydrophobic components, β -carotene and α -tocopherol, as well as chlorophyll-related compounds, such as pheophytins

[4,10,11]. These compounds also show strong antioxidative properties [5,12,13] and most are thought to remain in the waste of tea leaves after extraction with hot water. In fact, the waste of tea leaves showed antioxidative activity in chicks [14] and *in vitro* [15].

A hot water infusion of tea leaves, which is commonly ingested, includes a large amount of caffeine [8]. Caffeine is well known to be a central nervous system stimulant and causes insomnia, cardiac arrhythmia, and increases in blood cholesterol levels in animals [16]. It was reported that *ca.* 60% of the caffeine in tea leaves could be extracted by infusion with water at 100°C for 2 min [17], whereas only *ca.* 30% of catechins could be extracted under the same conditions [18]. Therefore, waste tea leaves are expected to include small amounts of caffeine and relatively large amounts of catechins and some hydrophobic antioxidants. Recently, canned and bottled green tea beverages have been produced industrially, and the manufacturing process results in a large mass of waste tea leaves. These waste tea leaves could be used as a natural decaffeinated source of antioxidants.

In this study, we prepared a low-caffeine extract from waste tea leaves and evaluated its antioxidative ability in mammals using male ddY mice.

2. Materials and Methods

2.1 Materials

Thiobarbituric acid (TBA) and α -tocopherol were purchased from Wako Pure Chemical Ind. (Osaka, Japan). β -Carotene and retinol were from Kanto Chemical Co. Inc. (Tokyo, Japan) and Sigma Chemical Co. (St. Louis, MO, U.S.A.), respectively. Tea catechins were obtained from Kurita Kogyo Co. (Tokyo, Japan). These vitamins and catechins were

used as standards for quantitative analysis. Other chemicals used were of reagent grade.

2.2 Preparation of aqueous extract from green tea leaves and extract from waste tea leaves

Samples of 20 g of commercial green tea leaves (Yabukita cultivar) were boiled in 2.5 L of water for 10 min and passed through filter paper. The filtrate was lyophilized to yield an aqueous extract of green tea leaves (green tea extract: GTE). Residual leaves were extracted with 750 mL of chloroform-methanol (2:1) mixture. The lower organic layer was collected and evaporated to obtain the low-caffeine tea extract (LCTE). The contents of catechins, gallic acid, and caffeine in prepared GTE and LCTE were determined by high-performance liquid chromatography (HPLC) [19]. The contents of β -carotene and α -tocopherol in GTE and LCTE were also determined by HPLC [20].

2.3 Determination of *in vitro* antioxidative activities of GTE and LCTE

With regard to GTE and LCTE, the antioxidative activities based on iron-chelating functions were determined by a method using autooxidation of mouse brain homogenates [21,22]. The radical scavenging activities of these extracts were also analyzed using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals [23].

2.4 Determination of *in vivo* antioxidative activities of GTE and LCTE

Male ddY mice, 5 weeks old, were obtained from Japan SLC, Inc. (Shizuoka, Japan) and divided into four groups. The mice in each group were given the following diets: (1) normal diet (control group); (2) normal diet supplemented with 1.0% GTE (1.0% GTE group); (3) normal diet supplemented with 0.5% LCTE (0.5% LCTE group); and (4) normal diet supplemented with 1.0% LCTE (1.0% LCTE group). The number of mice in each group was 10. Normal diet was a commercial powder, MF, from Oriental

Yeast Co., Ltd. (Tokyo, Japan). Diets and water were provided *ad libitum*. Mice were sacrificed under anesthesia with diethyl ether after 6 weeks of feeding the experimental or control diet. Throughout the experiment, the animals were handled in accordance with “*The Guide for Animal Experiments in the Numazu National College of Technology.*”

Body weight and food intake of mice were measured at various time points throughout the experimental period. After 6 weeks of feeding, the mouse liver was removed and homogenized in 19 volumes of 40 mM phosphate buffer (pH 7.4). Heparinized blood samples were taken and the plasma was prepared by centrifugation. Lipid peroxide contents as TBA reactive substances in mouse plasma were determined by the method of Masugi and Nakamura [24], and those in the liver were determined by the method of Yagi [25]. The contents are expressed in terms of malondialdehyde. Retinol and α -tocopherol contents in the plasma and liver of mice were determined by HPLC according to the method of Panfili *et al.* [20].

2.5 Statistical Analysis

Statistical analyses were performed with the non-parametric Mann-Whitney *U* test to determine the significance of differences between appropriate

experimental groups, and $P < 0.05$ was considered statistically significant.

3. Results and Discussion

3.1 Yields and components of GTE and LCTE

From 20 g of green tea leaves, 3.46 g of GTE and 2.04 g of LCTE were obtained. Therefore, the yields of GTE and LCTE were 17.3% (w/w) and 10.2% (w/w), respectively.

The contents of catechins, β -carotene, α -tocopherol, and caffeine in GTE and LCTE are shown in Table 1. The sums of the contents of the four major catechins in GTE and LCTE were 32.2% and 13.8% (w/w), respectively. The catechin contents in LCTE reached *ca.* 43% of that in GTE. β -Carotene and α -tocopherol were not detected in GTE, whereas the contents of both in LCTE were *ca.* 0.3% (w/w). When LCTE was added to the commercial diet at 0.5% or 1.0%, β -carotene contents in the diets were increased by 2.4- and 3.9-fold, respectively, and α -tocopherol contents were elevated by 1.1-fold and 1.3-fold, respectively. The caffeine content in LCTE, which was *ca.* 0.5% (w/w), was reduced to one-seventeenth that in GTE, 6.3% (w/w). In general, the content of caffeine in industrially prepared tea leaf products is *ca.* 1.5%, whereas that in normal green tea leaves is *ca.* 3.0%

Table 1 Contents of catechins, β -carotene, α -tocopherol, and caffeine in the aqueous extract of green tea leaves (GTE) and the low-caffeine extract of tea leaves (LCTE)

Components	Concentration (mg/g)	
	GTE	LCTE
(-)-Epicatechin	49.5	5.83
(-)-Epicatechin-3- <i>O</i> -gallate	24.8	12.5
(-)-Epigallocatechin	90.9	37.8
(-)-Epigallocatechin-3- <i>O</i> -gallate	156.7	82.3
Total content of catechins	321.9	138.4
β -Carotene	Not detected	3.10
α -Tocopherol	Not detected	2.97
Caffeine	63.3	4.56

[26]. It was suggested that GTE included concentrated caffeine as well as concentrated catechins. The content of caffeine in LCTE was much lower than that in tea leaves and GTE, while the catechin contents in LCTE were almost the same as those in tea leaves. Furthermore, LCTE included β -carotene and α -tocopherol. Waste tea leaves are thought to be available and convenient sources of low-caffeine food materials high in antioxidants.

3.2 *In vitro* antioxidative activities of GTE and LCTE

The inhibitory effects of GTE and LCTE on the autooxidation of mouse brain homogenates and scavenging activities of these extracts against DPPH radicals were evaluated. In the case of autooxidation of mouse brain homogenates, the 50% inhibitory concentrations (IC_{50}) of GTE and LCTE were 11.0 μ g/mL and 8.1 μ g/mL, respectively. The autooxidation was thought to be mainly due to iron, and the iron-chelating activities of LCTE were slightly stronger than those of GTE. In the case of DPPH radicals, IC_{50} values of GTE and LCTE were 0.128 mg/mL and 0.335 mg/mL, respectively. The radical scavenging activities of LCTE were slightly weaker than those of GTE. These results suggest that LCTE shows strong antioxidative activities similar to GTE.

3.3 Effects of diet supplemented with GTE and LCTE on body weight and food intake of mice

The body weights and food intake levels of the control, 1.0% GTE, 0.5% LCTE, and 1.0% LCTE groups are shown in Table 2. A decrease in body weight was observed previously in animals fed a diet supplemented with over 2.0% (w/w) tea polyphenol [27]. The total contents of tea catechins in 1.0% GTE, 0.5% LCTE, and 1.0% LCTE-supplemented diets were 0.32%, 0.069%, and 0.14% (w/w), respectively. Therefore, final body weights in the 1.0% GTE, 0.5% LCTE, and 1.0% LCTE group were similar to that of the control group in this study. Food intake levels in the 1.0% GTE, 0.5% LCTE, and 1.0% LCTE group showed no decreases in comparison with that of control group.

3.4 Effects of feeding the diets supplemented with GTE and LCTE on the lipid peroxide contents in the plasma and liver of mice

Lipid peroxide contents in the plasma and liver of the control, 1.0% GTE, 0.5% LCTE, and 1.0% LCTE groups are shown in Table 3. The contents of lipid peroxides in the plasma of 1.0% GTE, 0.5% LCTE, and 1.0% LCTE groups tended to be lower than those of control mice. The contents of lipid peroxides in the liver of 1.0% GTE, 0.5% LCTE, and 1.0% LCTE groups were significantly lower than those of the control group. Reduced glutathione is one of the most important antioxidative thiols in animal bodies and is a marker for oxidative stress *in vivo* [28]. In this study, the contents of reduced glutathione in the liver were

Table 2 Effects of feeding the diet supplemented with the aqueous extract of green tea leaves (GTE) and the low-caffeine extract of the tea leaves (LCTE) on body weight and food intake of ddY mice

Indexes	Control	1.0% GTE group	0.5% LCTE group	1.0% LCTE group
Numbers of mice	10	10	10	10
Initial body weight (g)	28.0 \pm 1.15	27.7 \pm 1.36	28.3 \pm 1.30	27.8 \pm 1.05
Final body weight (g)	34.7 \pm 3.28	34.6 \pm 1.77	34.1 \pm 1.57	34.7 \pm 2.59
Food intake (g/d/mouse)	4.66	4.92	4.85	4.64

Body weight is shown as the mean \pm SD.

Table 3 Effects of feeding the diet supplemented with the aqueous extract of green tea leaves (GTE) and the low-caffeine extract of the tea leaves (LCTE) on the contents of lipid peroxides in plasma and liver of ddY mice

Indexes	Control	1.0% GTE group	0.5% LCTE group	1.0% LCTE group
Plasma (nmol/mL)	10.37 ± 1.72	8.70 ± 3.53	9.86 ± 1.89	9.58 ± 2.03
Liver (nmol/g)	146.9 ± 11.0	124.1 ± 21.1*	123.6 ± 16.2*	114.8 ± 18.1*

Values are shown as the means ± SD. Significantly different from the control value: * $P < 0.05$.

measured by the method of Beutler *et al.* [29]. The contents of 0.5% LCTE and 1.0% LCTE groups were 1.4- and 1.5-fold higher than that of the control group, respectively. These differences between the contents of LCTE groups and the control group were statistically significant. This result indicated that oral intake of LCTE suppressed oxidative stress in the mouse liver.

3.5 Effects of diets supplemented with GTE and LCTE on the retinol and α -tocopherol contents in the plasma and liver of mice

As shown in Table 1, LCTE included large amounts of lipophilic antioxidants, β -carotene, and α -tocopherol in comparison with GTE. β -Carotene is known as provitamin A and is converted into vitamin A, retinol, in animal bodies. Administration of vitamin A enhances the resistance of human low-density lipoproteins [30] and the rat liver [31] and kidney [32] against lipid peroxidation *in vitro*. These antioxidative vitamins in LCTE are expected to inhibit lipid peroxidation in the plasma and liver of mice. The vitamin contents in the plasma of GTE and LCTE groups are shown in Table 4. No significant

differences were observed between these contents in the plasma of the control and 1.0% GTE groups. Retinol contents in the plasma of 0.5% and 1.0% LCTE groups were significantly higher than that of the control group. α -Tocopherol contents in the plasma of 0.5% and 1.0% LCTE groups were significantly higher than that of the control group. The increases in the contents of retinol and/or α -tocopherol are thought to be one of the reasons for the decrease in lipid peroxide contents observed in LCTE groups.

Considerable amounts of chlorophylls are included in tea leaves [4], and some chlorophyll-related compounds are known to have strong antioxidative activities [33,34]. We determined the chlorophyll content of LCTE by the method of Kitada *et al.* [4]. The level was 13.4 mg/g, which was thought to correspond to the major portion in tea leaves. Chlorophylls would partly contribute to the antioxidative effects of LCTE.

The results of the present study indicated that the LCTE-supplemented diet decreased lipid peroxide levels in the plasma and liver of mice, and LCTE is

Table 4 Effects of feeding the diet supplemented with the aqueous extract of green tea leaves (GTE) and the low-caffeine extract of the tea leaves (LCTE) on the contents of retinol and α -tocopherol in the plasma of ddY mice

Indexes	Control	1.0% GTE group	0.5% LCTE group	1.0% LCTE group
Plasma				
Retinol (ng/mL)	100.7 ± 18.1	116.0 ± 28.0	185.2 ± 67.3*	195.6 ± 14.2*
α -Tocopherol (μ g/mL)	6.77 ± 2.76	9.23 ± 2.84	15.9 ± 2.34*	11.0 ± 2.19*

Values are shown as the means ± SD. Significantly different from the control value: * $P < 0.05$.

expected to be useful as an antioxidative food additive for animals.

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緑茶葉から調製した低カフェイン抽出物の抗酸化作用

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要旨

緑茶の葉から調製した低カフェイン抽出物(LCTE)の抗酸化作用を、緑茶抽出物(GTE)と比較した。茶葉からの GTE と LCTE の収率は、17.3% (w/w)と 10.2% (w/w)であった。LCTE 中のカテキン含量は GTE の約 43%である一方、LCTE 中のカフェイン含量は GTE の約 8%と低かった。β-カロテンとα-トコフェロールは LCTE のみに検出され、その含量は約 0.3% (w/w)であった。*in vitro* の実験において、LCTE は GTE と同レベルの鉄キレート作用とラジカル捕捉作用を示した。LCTE の *in vivo* における抗酸化作用を検討するため、雄性 ddY 系マウスに市販の通常飼料、1.0% GTE を添加した飼料、0.5% LCTE を添加した飼料または 1.0% LCTE を添加した飼料の 4 種の飼料を 6 週間摂取させた。1.0% GTE、0.5% LCTE および 1.0% LCTE を添加した飼料の投与は、通常飼料を摂取させた場合に比較してマウス血漿中の脂質過酸化物量を低下させる傾向が見られた。マウスの肝臓では、1.0% GTE、0.5% LCTE および 1.0% LCTE 群の過酸化脂質量は通常飼料群と比較して有意に減少した。0.5%および 1.0% LCTE 添加飼料を摂取させたマウスの血漿中のレチノールおよびα-トコフェロールの濃度は、通常飼料を摂取させたマウスより有意に高かった。これらの結果から、LCTE の食事への添加は動物体内における酸化ストレスに対する抵抗性を高めるものと考えられる。

キーワード: 緑茶, 低カフェイン抽出物, 抗酸化, マウス