INHIBITORY EFFECTS OF JASMINE GREEN TEA EPICATECHIN ISOMERS ON FREE RADICAL-INDUCED LYSIS OF RED BLOOD CELLS

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Summary

Jasmine green tea is an excellent source of natural polyphenol antioxidants including mainly (-) epicatechin (EC), (-) epicatechin gallate (ECG), (-) epigallocatechin (EGC) and (-) epigallocatechin gallate (EGCG). The present study was to test our hypothesis that ingestion of jasmine tea would protect red blood cell (RBC) membrane from free radical-induced oxidation if jasmine tea epicatechin isomers could be absorbed and circulated in blood. When incubated with RBC suspension, all four epicatechin isomers purified from jasmine tea exhibited a strong protection for RBC membrane to hemolysis induced by 2,2'-azo-bis(2-amidinopropane) dihydrochloride (AAPH), an azo free radical initiator. The inhibitory effect was dose-dependent at the concentrations of 2.5 μM to 40 μM. The fatty acid analysis revealed that all four epicatechin isomers significantly prevented loss of arachidonic acid (20:4n-6) and docosahexaenoic acid (22:6n-3) in RBC incubated under the same conditions. Although the in vitro antioxidative activity of EGCG and ECG was more effective than EGC and EC, the latter two isomers were more important in vivo in scavenging free radicals. This was because only EGC and EC instead of EGCG and ECG were circulating in bloodstream after a gavage-dose of 100 mg jasmine tea GTP mixture. In fact, ingestion of jasmine tea GTP extracts was associated with a significant decrease in susceptibility of RBC to hemolysis in rats.

Key Words: jasmine tea, epicatechins, membrane oxidation, erythrocyte

Activated oxygen species are thought to involve in the damage of biomembrane during ischemia, inflammation, aging, β-thalassaemia, sickle cell anaemia and glucose -6-phosphate dehydrogenase deficiency (1-3). It has been shown that low vitamin E in red blood cell (RBC) membrane is associated with the increased susceptibility to hemolysis (4,5). Several antioxidants including α-tocopherol and trolox have been demonstrated to be effective against RBC hemolysis (5-7).

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Jasmine green tea is one of the most popular beverages consumed in China. We have previously found that jasmine green tea is an excellent source of polyphenol antioxidants including mainly (-) epicatechin (EC), (-) epicatechin gallate (ECG), (-) epigallocatechin (EGC) and (-) epigallocatechin gallate (EGCG). We have been able to demonstrate that these epicatechins purified from jasmine green tea inhibit oxidation of frying canola oil more effectively than butylated hydroxytoluene (BHT). Furthermore, we have shown that jasmine green tea polyphenol (GTP) extracts and its epicatechin isomers are effective agents to protect human low-density lipoprotein from oxidative modification.

In view of the report by Serafini et al (10), who showed that the ingestion of tea produced a significant increase in human plasma antioxidant capacity in vivo, we hypothesize that the ingestion of jasmine tea GTP would strengthen the protection of red blood cell membrane from free radical-induced oxidative damage if green tea epicatechins can be absorbed and circulated in blood. The present study was therefore to examine the circulation of green tea epicatechins in plasma and their effects on RBC hemolysis induced by an azo free-radical initiator after a gavage-dose of jasmine tea GTP extracts in rats. We also sought to ascertain whether oxidation of polyunsaturated fatty acid species in RBC membrane might be prevented by jasmine tea epicatechins.

**Material and Methods**

**HPLC analysis of jasmine tea GTP extracts.** The method described by Agarwal et al (11) was used to extract total GTP from jasmine tea. The individual isomers in jasmine tea extracts were separated using a Shimazu LC-10AD HPLC (Tokyo, Japan) equipped with a ternary pump delivery system. In brief, 15 μL of jasmine tea extracts (2 mg/mL) was injected onto column (Hypersil ODS, 250×4.6 mm, 5 μm, Alltech, Deerfield, IL, USA) via a rheodyne valve (20 μL capacity, Shimadzu, Tokyo, Japan). An eluting mixture of H₂O containing 0.05 % H₂SO₄, acetonitrile and ethyl acetate (86:12:2, vol/vol/vol) was used at a flow rate of 1 mL/min. The separated GTP isomers were monitored using a UV detector at 280 nm (UVIS-205, Alltech, Deerfield, IL, USA) and quantified using (+) catechin as an internal standard (Fig. 1). Identification of each isomer was confirmed by comparison of retention time and co-chromatography with authentic standards of EC, ECG, EGC and EGCG (Kurita Industrial Co., Ltd, Tokyo, Japan).

**Isolation and purification of individual GTP isomers.** Individual jasmine tea GTP isomers were isolated using a semipreparative column (Spherisorb ODS-1, 250×10 mm, 10 μm, Isco, Inc., Lincoln, NE, USA). In brief, 50 mg jasmine tea GTP extracts in H₂O was loaded onto the column via a rheodyne valve with a 250 μL sample loop. A 29% methanol solution in H₂O was used at a flow rate of 0.7 mL/min. The eluting peaks of epicatechin isomers were monitored at 280 nm using a UV detector (UVIS-205, Alltech, Deerfield, IL, USA) and quantified using (+) catechin as an internal standard (Fig.1). Identification of each isomer was confirmed by comparison of retention time and co-chromatography with authentic standards of EC, ECG, EGC and EGCG (Kurita Industrial Co., Ltd, Tokyo, Japan).

**In vitro study of anti-hemolysis activity of jasmine tea GTP extracts and epicatechin isomers.** Male Sprague-Dawley rats (300 g) were fed a rodent chow diet ad libitum (Ralston Purina, St Louis, MO, USA) for a week. Blood (8-10 mL/rat) from abdominal aorta was collected into
a heparinized tube. The RBC was separated from plasma by centrifugation at 1,500 g for 20 min. The crude RBC was then washed three times with 5 volumes of phosphate buffered saline (PBS, pH=7.4). The packed RBC was thereafter suspended in 4 volumes of PBS solution.

The oxidative hemolysis in RBC induced by various agents including hydrogen peroxide (12,13), dialuric acid (14,15), xanthine oxidase (16,17), and 2,2'-azo-bis(2-amidinopropane) dihydrochloride (AAPH; 6,7) has been extensively studied as a model for the peroxidative damage in biomembrane. In the present study, the method described by Miki et al (6) was used to determine the hemolysis of RBC mediated by AAPH. Addition of AAPH (a peroxyl radical initiator) to suspension of RBC caused oxidation of lipids and proteins in cell membrane and thereby induce hemolysis. It has been known that the AAPH-induced hemolysis in RBC is a function of incubation time and is proportional to the concentration of free radicals (6,7). The inhibitory effect on RBC hemolysis is also proportional to the concentration of antioxidants in the incubation mixture. Two mL of RBC suspension was mixed with 2 mL PBS solution containing varying amounts of jasmine tea GTP and its epicatechin isomers (2.5 - 40 μM). Two mL of 200 mM AAPH in PBS solution was then added to the mixture. The incubation mixture...
was shaken gently in a water bath at 37°C for 3 hours. After incubation, 8 mL of PBS solution was added into the reaction mixture followed by centrifugation at 1,000 g for 10 min. The absorbance (A) of the supernatant at 540 nm was recorded in a Shimazu 1201 spectrophotometer. Percentage inhibition was calculated by the following equation.

\[
\% \text{ Inhibition} = \frac{[A_{\text{AAPH}} - A_{\text{GTP}}]}{A_{\text{AAPH}}} \quad \text{equation 1}
\]

Where: \( A_{\text{GTP}} \) is the absorbance of the sample containing jasmine tea GTP or epicatechin isomers, and \( A_{\text{AAPH}} \) is the absorbance of the control sample containing no jasmine GTP or epicatechin isomers.

To follow the change in polyunsaturated fatty acids of RBC membrane, an additional set of RBC suspension was incubated under the same conditions. After incubation, two volumes of distilled H₂O and 100 μL of 100 mM butylated hydroxytoluene solution were added. After 20 min, the incubation mixture was centrifuged at 1,500 g and the supernatant was removed. The lipids of cell membrane were thereafter extracted using chloroform/methanol (2:1, vol/vol) containing 0.02% butylated hydroxytoluene as an antioxidant. The lipid extracts were then converted to methyl esters by using a mixture of 14% BF₃ in methanol and toluene (2:1, vol/vol) under nitrogen at 90°C for 45 min. The fatty acids methyl esters were analyzed by gas liquid chromatography using a SP-2560 flexible fused silica capillary column (100 m × 0.25 mm I.D., 20 μm film thickness; Supelco, Inc. Bellefonte, PA, USA) in a Hewlett-Packard 5890 Series II gas chromatograph equipped with a flame ionization detector (Palo Alto, CA, USA). The column temperature was programmed from 180 to 220°C at rate of 1°C/min and then held for 20 min. Injector and detector temperatures were set at 250°C and the column head pressure was set at 15 PSI.

**In vivo study of anti-hemolysis activity of jasmine tea GTP extracts.** Male Sprague-Dawley rats (300 mg) were randomly divided into two groups. One group was gavage-dosed with one mL of distilled water containing 100 mg jasmine tea GTP extracts (GTP-G) whereas the control group was gavage-dosed with one mL of distilled water containing no jasmine tea GTP extracts (CTL-G). After 20 min, the blood from aorta was collected into a heparinized tube. The RBC was separated from plasma by centrifugation at 1,500 g for 20 min. After removal of white blood cells and platelets, the remaining RBC was combined with the volume of plasma therefrom. The reconstituted blood (without white blood cells and platelets) was then subject to the hemolysis assay by adding 2 mL of AAPH solution and 2 mL of PBS followed by incubation at 37°C for 1, 3, and 4 hours, respectively. As described above, 8 mL of PBS solution was added into the incubation mixture followed by centrifugation at 1,000 g for 10 min. The absorbance (A) of the supernatant at 540 nm was measured and the percentage inhibition of a gavage-dose of jasmine tea GTP was calculated by the same way as equation 1.

\[
\% \text{ Inhibition} = \frac{[A_{\text{CTL}} - A_{\text{GTP-G}}]}{A_{\text{CTL}}} \quad \text{equation 2}
\]

Where: \( A_{\text{GTP-G}} \) is the absorbance of the reconstituted blood obtained from rats orally administered with 1 mL of distilled water solution containing 100 mg jasmine tea GTP extracts, and \( A_{\text{CTL}} \) is the absorbance of the reconstituted control blood samples obtained from the control rats.

To investigate the absorption and circulation of epicatechin isomers after a gavage dose of 100 mg jasmine tea GTP extracts, the rats were killed at 10, 20, 30, 40 and 60 minutes. The aorta blood was collected and 4 mL of plasma was extracted twice with 4 mL of ethyl acetate. The ethyl acetate was then removed under a gentle stream of nitrogen. The residues
were then dissolved in 200 µL of ethyl acetate and subject to HPLC analysis as described above in the section of HPLC analysis of jasmine tea GTP extracts.

Statistics. Data for hemolysis inhibition and fatty acid analysis were subjected to the analysis of variance (ANOVA), and the means were compared between treatment by using Duncan’s multiple range test. This was done by running data on the PC ANOVA software (PC ANOVA for the IBM Personal Computer, Version 1.1, 1985; IBM, Armonk, NY).

Results

The absolute and relative composition of jasmine tea GTP is shown in Table 1. These polyphenols present in jasmine tea were mainly epicatechin isomers including EGCG, EGC, EC and ECG. The chemical structures of these epicatechin isomers are characterized by sharing a similar backbone with varying number and location of hydroxyl groups (Fig. 2). The composition of jasmine tea epicatechin isomers varies with batches. HPLC analysis showed that the yield of jasmine tea GTP was 7.5 g/100 g dry jasmine tea leaves from three determinations (Table 1). In the batch used in the present study, EGCG was the major isomer and accounted for 62.3% followed by ECG (19.2%), EGC (8.3%) and EC (4.6%).

![Chemical structures of (-) epigallocatechin (EGC), (-) epigallocatechin gallate (EGCG), (-) epicatechin (EC), and (-) epicatechin gallate (ECG).](image)

To study the relative antioxidative activity of individual epicatechin isomers, four epicatechin isomers were separated and purified from jasmine tea extracts. All four epicatechin isomers isolated from jasmine tea GTP extracts in vitro inhibited AAPH-induced hemolysis at
the concentrations ranging from 2.5 μM to 40 μM with varying effectiveness (Fig.3). The inhibitory effect of EGCG and ECG was much stronger than that of EGC and EC. At the concentrations of 5 and 10 μM, the inhibitory effect of EGCG and ECG was almost two times higher than that of EGC and EC (p < 0.01).

Table 1.
Composition of jasmine tea epicatechin isomers

<table>
<thead>
<tr>
<th>Polyphenol isomers</th>
<th>Relative (%)</th>
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<tbody>
<tr>
<td>(-) Epigallocatechin gallate (EGCG)</td>
<td>62.3±0.4</td>
</tr>
<tr>
<td>(-) Epicatechin gallate (ECG)</td>
<td>19.2±0.2</td>
</tr>
<tr>
<td>(-) Epigallocatechin (EGC)</td>
<td>8.3±0.1</td>
</tr>
<tr>
<td>(-) Epicatechin (EC)</td>
<td>4.6±0.1</td>
</tr>
<tr>
<td>Others</td>
<td>5.6±0.2</td>
</tr>
<tr>
<td>Total GTP (g/100g dry tea leaves)</td>
<td>7.5±0.3</td>
</tr>
</tbody>
</table>

*Data are expressed as means ± SD (n=3).

Inhibitory effect of individual jasmine tea epicatechin isomers on AAPH-induced lysis of rat red blood cells in vitro. EGC, (-) epigallocatechin; EGCG, (-) epigallocatechin gallate; EC, (-) epicatechin; ECG, (-) epicatechin gallate (ECG). Data are expressed as mean of n = 5-6 samples. a, b, c Means at the same dose point with different superscript letters differ significantly at p < 0.01.
The *in vitro* effect of jasmine tea GTP extracts and four epicatechin isomers on the changes in RBC membrane fatty acids during AAPH-induced hemolysis was shown in Table 2. The loss of arachidonic acid (20:4n-6) and docosahexaenoic acid (22:6n-3) in the RBC with addition of jasmine tea GTP and its individual epicatechin isomers was significantly less compared with that in the control. Under the same conditions, 18:0, 16:0 and 18:1 n-9 in the RBC were proportionally increased in the RBC incubated in the absence of jasmine tea GTP extracts or individual epicatechin isomers. Although the hemolysis test demonstrated that EGCG and ECG were more effective than EGC and EC against oxidation of the RBC membrane induced by AAPH, the fatty acid analysis failed to detect any difference. This indicated that the hemolysis test was more sensitive than the fatty acid analysis method in detecting antioxidant activity of epicatechin isomers on biomembrane.

<table>
<thead>
<tr>
<th>Table 2</th>
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<tbody>
<tr>
<td>Effects of jasmine green tea polyphenol (GTP) extracts (16 µg/mL) and individual epicatechin isomers (40 µM) on the change in major fatty acids (wt% of total fatty acids) of red blood cell (RBC) membrane incubated with 2,2'-azo-bis(2-amidinopropane) dihydrochloride (AAPH)*.</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th></th>
<th>16:0</th>
<th>18:0</th>
<th>18:1n-9</th>
<th>18:2n-6</th>
<th>20:4n-6</th>
<th>22:6n-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unoxidized RBC</td>
<td>25.6±0.6*</td>
<td>27.1±1.0*</td>
<td>4.3±0.2*</td>
<td>8.0±0.3*</td>
<td>17.0±0.9*</td>
<td>3.8±0.3*</td>
</tr>
<tr>
<td>RBC+AAPH</td>
<td>30.5±0.8*</td>
<td>30.4±1.4*</td>
<td>6.3±2.8*</td>
<td>5.6±0.8*</td>
<td>9.5±2.0*</td>
<td>1.9±0.2*</td>
</tr>
<tr>
<td>RBC+AAPH+GTP</td>
<td>27.8±0.8*</td>
<td>30.8±1.1*</td>
<td>5.3±0.3*</td>
<td>6.4±0.5*</td>
<td>13.6±1.0*</td>
<td>3.4±0.4*</td>
</tr>
<tr>
<td>RBC+AAPH+EGC</td>
<td>29.7±2.9*</td>
<td>29.4±1.2*</td>
<td>5.1±0.4*</td>
<td>6.8±0.8*</td>
<td>13.1±0.3*</td>
<td>2.8±0.1*</td>
</tr>
<tr>
<td>RBC+AAPH+EGCG</td>
<td>28.6±1.3*</td>
<td>30.9±2.2*</td>
<td>5.4±2.0*</td>
<td>6.8±0.6*</td>
<td>13.2±1.0*</td>
<td>2.7±0.7*</td>
</tr>
<tr>
<td>RBC+AAPH+EC</td>
<td>28.6±0.3*</td>
<td>30.3±1.7*</td>
<td>5.9±2.3*</td>
<td>5.8±0.3*</td>
<td>13.1±0.8*</td>
<td>2.6±0.3*</td>
</tr>
<tr>
<td>RBC+AAPH+ECG</td>
<td>28.5±0.5*</td>
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<td>13.9±0.7*</td>
<td>2.8±0.9*</td>
</tr>
</tbody>
</table>

*Data are expressed as mean ± SD of n = 5-6.

*<sup>a</sup>,<sup>b</sup>,<sup>c</sup> means at the same column with different superscripts differ significantly at p<0.05.

EGC, (-) epigallocatechin; EGCG, (-) epigallocatechin gallate; EC, (-) epicatechin; ECG, (-) epicatechin gallate.

When the GTP-G rats were orally administered with 100 mg jasmine tea GTP as a mixture, several additional peaks were detected in aorta blood compared with CTL-G rats (Fig.4). Although LC-MS (liquid chromatography-mass spectrophometry) was not used in the present study, the retention time and UV spectrum of these peaks indicated that only EGC and EC but not EGCG and ECG were possibly circulated in aorta blood. The time-course change in concentrations of EGC and EC in plasma was graphically illustrated in Figure 5. The concentration of EGC sharply increased to 6 µg/mL plasma and, thereafter, remained unchanged until 60 min after an oral dose of 100 mg jasmine tea GTP extracts. Under the same conditions, the concentration of EC in plasma peaked at 40 min.

The results of the *in vivo* tests are presented in Figure 6. The RBC in the reconstituted blood (without white blood cells and platelets) obtained from GTP-G rats was more resistant to AAPH-induced hemolysis than that obtained from CTL-G rats (Fig.6). Together with the results of *in vitro* study, this suggests that jasmine tea epicatechins as antioxidants inhibit AAPH-induced hemolysis of RBC both *in vitro* and *in vivo*.  

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**Jasmine Green Tea on RBC**
Typical HPLC chromatograms of ethyl acetate extracts of plasma from rat aorta blood 20 min after an oral ingestion of one mL distilled water containing 100 mg jasmine tea GTP extracts (GTP-G, top) and one mL of distilled water containing no jasmine GTP extracts (CTL-G, bottom). EGC, (-) epigallocatechin; EC, (-) epicatechin.

**Discussion**

The biomembrane may be most susceptible to free-radical attack due to its content of polyunsaturated fatty acids. To our best knowledge, the present study was the first report to demonstrate that jasmine tea epicatechins as a mixture or separately *in vitro* protect these polyunsaturated fatty acids from oxidation in the membrane of RBC incubated with AAPH. The fatty acid analysis revealed that the extensive hemolysis of RBC caused by AAPH was associated with a significant decrease in 20:4n-6 and 22:6n-3 (Table 2). Addition of jasmine tea GTP or individual epicatechin isomers significantly prevented loss of 20:4n-6 and 22:6n-3 in RBC incubated under the same conditions. However, the result of the present study was in
Fig. 5
Change in concentrations of epicatechin isomers after a gavage-dose of 100 mg jasmine tea GTP extracts in rats. Each value is expressed as mean ± SD of n = 5-6 rats.

Fig. 6
The time course AAPH-induced hemolysis of the reconstituted red blood cells (without white blood cells and platelets) obtained from rats 20 min after an oral ingestion of one mL of distilled water containing 100 mg jasmine tea GTP extracts (GTP-G) or containing one mL of distilled water only (CTL-G). Data are expressed as mean ± SD of n = 5-6 rats. *Means at the same time point differ significantly between GTP-G and CTL-G rats at p < 0.01.
contrast to that of Miki et al. (6) who observed that the hemolysis of RBC mediated by AAPH was only associated with a decrease in 20:4n-6 but with 22:6n-3 being unchanged. This discrepancy may arise from the different experimental conditions in the study of Miki et al. (6) who used a packed GC column in the fatty acid analysis of RBC membrane.

Jasmine tea epicatechins as a GTP mixture or separately possess a strong inhibitory effect on free radical-induced hemolysis of RBC in vitro. In this regard, the protection of jasmine tea GTP and four epicatechin isomers to RBC membrane was dose dependent with maximum effect at the concentration of 16 µg/mL GTP (data not shown) or 40 µM epicatechin isomers in vitro (Fig.3). The resistance of biomembrane to oxidation is positively associated with the content of antioxidants in or surrounding the membrane (6,7). The present study was in agreement with that of Nanjo et al. (18) who showed that addition of 1 % green tea catechins in diet significantly prevented the lipid oxidation of RBC and loss of α-tocopherol in rats fed on perilla oil diets. Similar data were also obtained by Miki et al. (6) who showed that extent of hemolysis was related to the loss of α-tocopherol during AAPH-induced hemolysis.

Jasmine tea GTP extracts and its individual epicatechin isomers may protect RBC membrane from free radical attack by either one or combination of following mechanisms. First, jasmine GTP extracts or individual epicatechin isomers act as a chelator to inactivate Cu^{2+} and catalytic cations involved in initiation of free radicals (19). Second, they may function as a free-radical chain reaction interrupter by trapping the free radicals mediated by AAPH. Third, they may function synergistically with α-tocopherol by donating a hydrogen to regenerate α-tocopherol when the latter was oxidized (19-22).

When the in vitro relative activity of four epicatechin isomers in jasmine tea as antioxidants against AAPH-induced hemolysis was compared, EGCG and ECG were more effective against AAPH-induced hemolysis than EGC and EC. Similar data have been obtained in a separate study which showed that EGCG and ECG were stronger than EGC and EC in protection of low-density lipoprotein from oxidation (9). The varying effect of individual epicatechins on AAPH-induced membrane oxidation was probably related to the number and position of their hydroxyl groups. An additional gallate group in EGCG and ECG increases total number of phenol hydroxyl groups. For one thing, it makes EGCG and ECG more vulnerable to donate a hydrogen than EGC and EC; for another, it makes EGCG and ECG more hydrophilic and therefore more chelating power to catalytic cations such as Cu^{2+}.

It is noteworthy that jasmine tea epicatechins have in vitro antioxidative activity. However, it does not guarantee their viability in vivo if jasmine tea epicatechins can not be absorbed and circulated in blood. To prove the antioxidative activity of jasmine tea epicatechins in vivo, we have been able to show that jasmine tea epicatechins are absorbed at least in part. However, only EGC and EC were circulated in aorta blood (Fig.4 & 5). This may be explained by either one or combination of following possibilities. First, absorption of EGC and EC may be more efficient that of EGCG and ECG. Second, EGCG and ECG may be hydrolyzed to form EGC and EC, respectively, before they are absorbed (Fig.2). Third, all four isomers may be absorbed via portal vein to liver (23) where EGCG and ECG may be hydrolyzed to form EGC and EC, respectively. Nevertheless, the mechanisms by which EGC and EC are circulated in blood but not EGCG and ECG remains a mystery and deserves further study.

Jasmine tea contains lesser amounts of EGC and EC than their corresponding gallate derivatives, EGCG and ECG (Table 1). Although the in vitro antioxidative activity of EGC and EC were less effective than EGCG and ECG (Fig.3), the former two isomers were more important in vivo than the latters because EGC and EC were only two isomers circulating in blood after ingestion of jasmine tea GTP extracts. If ingestion of green tea is significantly associated with a decrease in susceptibility of RBC to hemolysis and other free-radical related diseases, part of the mechanisms may involve circulation of EGC and EC as free radical
scavengers in blood stream. The present data supported the recent study by Serafini et al (10), who have shown that drinking tea elicits a rise in vivo of plasma antioxidative defence. In addition, ingestion of green tea also protects human low-density lipoproteins from oxidative modification (9).

In conclusion, the present results suggest that Chinese jasmine tea contains a significant amount of polyphenol antioxidants which are mainly isomers of epicatechin. These epicatechin isomers as a GTP mixture or separately are effective antioxidants both in vitro and in vivo in protection of RBC membrane to oxidation. It was the first time to observe that only EGC and EC but not EGCG and ECG were present in blood after an oral ingestion of jasmine tea GTP extracts. The present study suggests that the active compounds are most likely EGC and EC instead of their gallate derivatives, EGCG and ECG, although the former two isomers are quantitatively minor in green tea leaves.

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References