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In vitro antioxidant and anticancer activities of ethanolic extract of selenium-enriched green tea

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Selenium-enriched green tea is now being increasingly produced in China and is well known as a bioactive beverage, due to its high content of active components. In this study, the antioxidant and anticancer activities of an ethanolic extract and an aqueous extract of Se-enriched green tea were investigated. The results indicated that the ethanolic extract possessed significantly higher antioxidant activity than the aqueous extract and the positive control α-tocopherol, by both α,α-diphenyl-β-picrylhydrazyl (DPPH) radical-scavenging and ferric thiocyanate (FTC) assays. The ethanolic extract inhibited the proliferation of human cervical adenocarcinoma HeLa cell and possessed a significantly higher antitumour activity than the aqueous extract and the positive control 5-fluorouracil (5-FU), in the dose range of 62.5–250 μg/ml. Moreover, the ethanolic extract could significantly inhibit the growth of lung carcinoma A549 and hepatoma HepG2 in a concentration-dependent manner, with IC_{50} values of 278.6 μg/ml and 431.6 μg/ml, respectively. Selenium, tea polyphenols and polyphenols constituents, especially epigallocatechin gallate (EGCG), were significantly higher in the ethanolic extract than in the aqueous extract, which were possibly responsible for the higher antioxidant and antitumour activities of the ethanolic extract.

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1. Introduction

Selenium has received considerable attention as an essential micronutrient for humans and animals. Selenium plays an essential role in the formation of glutathione peroxidases (GSH-Px), thioredoxin reductase (TRx), iodothyronine deiodinases, selenophosphate synthetase, selenoprotein P and other selenoproteins (Letavayova, Vlckova, & Brozmanova, 2006). Glutathione peroxidases and thioredoxin reductase have been reported to be associated with its antioxidant activity, anticancer effect, and other physiological functions (Arteel & Sies, 2001; Letavayova et al., 2006). Selenoprotein P appeared to protect the endothelial cells against damage from free radicals (Allan, Lacourciere, & Stadtman, 1999; Ganther, 1999).

Tea is one of the most widely consumed beverages in the world. At present, Se-enriched green tea is being increasingly produced in China and is well known as a bioactive drink, due to its high content of active components (Xu, Yang, Chen, Hu, & Hu, 2003).

Active oxygen and free radicals are increasingly being recognised as being responsible for the pathogenesis of certain human diseases, including cancer, aging and chronic arterial disease (Moskovitz, Yim, & Chock, 2002). A number of epidemiological studies, though inconclusive, have shown that the aqueous extract of green tea and selenium compounds were potential cancer chemopreventive agents (Cao, Durrani, & Rustum, 2004; Park, Han, Park, & Park, 2005). Farhoosh's research found a high antioxidant activity for hot water extract of green tea leaves (Farhoosh, Golmovahhed, & Khodaparast, 2007). Steele's report provided a strong evidence of antimutagenic, antiproliferative and antineoplastic activity for an aqueous extract of green tea (Steele et al., 2000). Most of these effects have been attributed to the antioxidant and free radical-scavenging properties of tea, particularly to its high contents of polyphenolic compounds and microelements (Yang, Sang, et al., 2006). The chemopreventive and antitumour effects of green tea polyphenols (GTP), particularly (−)-epigallocatechin gallate (EGCG), which is the most abundant and biologically active catechin, have been studied extensively in chemically-induced animal tumour models, as well as several types of cancer cells in culture (Chen, Yu, Owuor, & Kong, 2000; Katiyar, Elmets, & Katiyar, 2007; Na & Surh, 2006; Stuart, Scandlyn, & Rosengren, 2006; Yang, Lambert, et al., 2006).

The contents of the bioactive components in the extracts of Se-enriched tea vary with different extraction methods. Water, aqueous mixtures of ethanol, methanol and acetone are commonly used to extract bioactives from plants (Sun & Ho, 2005). In our previous study, Se-enriched green tea was extracted with hot water, and the aqueous extracts exhibited high antioxidant activity (Xu,

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Yang, et al., 2003; Xu, Zhu, Yang, Cheng, & Hu, 2003). However, studies have shown that extraction with mixed solvents gave extracts with considerably higher antioxidant activity than those with single solvents (Spigno, Tramelli, & De Faveri, 2007; Turkmen, Sari, & Velioglu, 2006).

In the present study, we evaluated an ethanolic extract with an aqueous extract of Se-enriched green tea for their *in vitro* antioxidant activity by DPPH radical-scavenging and ferric thiocyanate (FTC) methods, and anticancer activity, using MTT assay. Furthermore, in order to understand the relationship between bioactive properties and antioxidant and antitumour activities, the constituents of the ethanolic and aqueous extracts of Se-enriched green tea were analysed.

2. Materials and methods

2.1. Chemicals

Epigallocatechin (EGC), DL-catechin (DL-C), epicatechin (EC), epigallocatechin gallate (EGCG), epicatechin gallate (ECG), α,α-diphenyl-β-picrylhydrazyl (DPPH), α-tocopherol, acetic acid and acetonitrile of chromatographic grade were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO). Dulbecco modified Eagle's medium, penicillin and streptomycin were obtained from Invitrogen (CarIsbad, CA). Linoleic acid (99%) and 2,2-azobis(2-amidinopropane)dihydrochloride (AAPH) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Fluka, USA. Fetal bovine serum (FBS) was obtained from Sijiqing Biological Engineering Material Co., Ltd. (Hangzhou, China). 5-Fluorouracil (5-FU) was purchased from Nantong Pharmacy Factory (Nantong, China). Other reagents were of analytical grade and purchased from Nanjing Chemical Industry (Nanjing, China).

2.2. Preparation of extracts of Se-enriched green tea

2.2.1. Ethanolic method

The Se-enriched green tea was prepared according to the protocols reported previously (Hu, Xu, & Pan, 2003). Subsequently, dry tea was milled into powder of 80 mesh particle size, and was stored at −20 °C. The conditions for ethanol extraction of Se-enriched green tea was optimised by an orthogonal experiment reported previously (Wang, Yu, Xin, & Hu, 2007). Briefly, 10 g of Se-enriched green tea powder was extracted with 150 ml of 50% (v/v) ethanol at 50 °C, assisted by sonication (250 W) for 1.5 h. Each sample was extracted in duplicate under the same conditions. After being filtered, the supernatant was then combined and concentrated in a rotary evaporator under vacuum, lyophilised, and stored at −20 °C for further assay.

2.2.2. Hot water method

The traditional method of extraction, aqueous extraction, was conducted by adding 150 ml of distilled water to 10 g Se-enriched green tea powder in a flask, followed by steeping in a water bath at 100 °C for 2 h. The mixture was filtered and the residue was extracted in duplicate under the same conditions. Subsequently, the filtrates were combined and evaporated under vacuum, lyophilised, and stored at −20 °C until further assay.

2.3. Determination of antioxidant activity with the DPPH radical-scavenging activity method

The radical-scavenging capacity of α-tocopherol, ethanolic and aqueous extracts of Se-enriched green tea was determined using the DPPH radical method (Sheng, Zhou, Wang, Xu, & Hu, 2007). A 2 ml aliquot of each solution (50 μg/ml) was added to 2 ml of 2×10^{-4} mol/L ethanolic DPPH solution in a cuvette. The mixture was shaken vigorously and the absorbance was measured at 517 nm immediately. The decrease in absorbance was determined at 15 and 30 min until the absorbance reached a steady state (after nearly 30 min). The mixture with the addition of α-tocopherol served as a positive control. All the tests were performed in triplicate, and the inhibition rate was calculated according to the formula of Yen and Duh (1994).

2.4. Determination of antioxidant activity with ferric thiocyanate (FTC) method

The antioxidant activity of α-tocopherol, ethanolic and aqueous extracts of Se-enriched green tea was assayed using a linoleic acid system. One millilitre of 50 μg/ml sample, 2 ml of 2.51% (w/v) linoleic acid in ethanol, 4 ml of 0.05 M phosphate buffer (pH 7.0), and 2 ml of distilled water were mixed in a tube of 10 ml with a screw cap. The oxidation was initiated by the addition of 0.417 ml of 0.1 M 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH) and then kept in a 37 °C water bath in the dark. The above mixture (0.1 ml) was added to 9.7 ml of 75% (v/v) ethanol and 0.1 ml of 30% (w/v) ammonium thiocyanate. After 5 min, 0.1 ml of 0.02 M ferrous chloride in 3.5% (v/v) hydrochloric acid was added to the above mixture and then mixed. The absorbance of the mixture was recorded at 50 min, 100 min and 200 min at 500 nm. The degree of oxidation was measured according to the ferric thiocyanate (FTC) method, described in detail by Kikuzaki and Nakatani (1993). Linoleic acid mixture without the addition of sample was used as the control and α-tocopherol at the same concentration served as the reference antioxidant.

2.5. Cell lines and cell culture

Human cervical adenocarcinoma HeLa, human lung carcinoma A549 and human hepatoma HepG2 cell lines were obtained from the Cell Bank of Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences (Shanghai, China). The cells were grown in 90% DMEM supplemented with 10% FBS, 100 U/ml penicillin, and 100 μg/ml streptomycin at 37 °C in a humidified atmosphere of 5% CO_2.

2.6. Cell proliferation assay

To screen out an extract of Se-enriched green tea with high antioxidant and anticancer activities, a preliminary trial was performed based on HeLa cell proliferation exposed to various concentrations of ethanolic and aqueous extracts of Se-enriched green tea. Furthermore, to evaluate *in vitro* inhibition of the extract with higher bioactivity against cancer cells, a verification experiment was conducted with A549 and HepG2 cells by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay with some slight modifications (Mosmann, 1983).

Briefly, logarithmically growing cells were seeded in 96-well culture plates (2×10^5 cells/well) for 24 h at room temperature, then exposed to various concentrations of samples in an incubator with 5% CO_2 at 37 °C for 48 h. The culture medium was removed and MTT reduction was initiated by adding 20 μl MTT solution (5 mg/ml) per well. After 4 h incubation, the supernatant was discarded and 100 μl dimethyl sulfoxide was added to each well to terminate the reaction. The mixture was shaken and optical density was measured at 570 nm using a Universal Microplate Reader (EL800, BIO-TEK Instruments, USA). Treatment with 5-FU at the same concentration served as the positive control. All samples were assayed in triplicate and cell survival was expressed as a

percentage of the control, which was considered to be 100%. The IC_{50} value was calculated as the sample concentration that caused 50% cell death by using SPSS statistical software (version 13.0).

2.7. Analysis of constituents of Se-enriched green tea extracts

Contents of Se, tea polyphenol (TPP), tea polysaccharide (TPS) and protein were determined, according to the method of Yu, Sheng, Xu, An, and Hu (2007), with minor modifications.

Se content was analysed by dual-hydride generation atomic fluorescent spectrometry (AFS-3100, Beijing Kechuang Haiguang Instrument, Beijing, China). The powder samples were digested with 10 ml of a mixture of HNO_3 and $HClO_4$ (v/v, 4:1) at 170–175 °C for 45 min. After cooling, 5 ml of concentrated HCl was added, to reduce Se^{6+} to Se^{4+} and continued for an additional 30 min, until the samples were completely mineralised. The digest solution was diluted to 50 ml with distilled water. Ten millilitres of the solution was transferred to a reaction vessel; 2 ml of concentrated HCl and 1 ml of 10% (w/w) $K_3Fe(CN)_3$ were added, prior to determination with an atomic fluorescence spectrometer.

TPP contents were measured by ferrous tartrate colorimetry. TPS contents were determined by phenol-sulfuric acid methods, using glucose as the standard. The protein content was analysed by the Lowry–Folin method (Lowry, Rosebrough, Farr, & Randall, 1951).

2.8. Component analysis of tea polyphenol

The components of tea polyphenols were analysed by HPLC (Waters 600, Waters Corporation, Milford, MA) with Waters 2487 dual wavelength absorbance detector at 280 nm, using a Hypersil ODS column (100 × 4.6 mm i.d., particle size 3 μm, pore size 120 Å; Phenomenex, Torrance, CA) (Yang & Raner, 2005). A mixture of acetic acid (2%) and acetonitrile, of which acetic acid varied linearly from 92% to 82.7% in 20 min, was employed as mobile phase at a flow rate of 1.2 ml/min. Five individual catechins were quantified in both extracts, by comparison with the generated standard curves.

2.9. Statistical analysis

The data were presented as means ± standard deviations of three determinations. Statistical analysis was performed using Student's *t*-test and one-way analysis of variance. Multiple comparisons of the means were done by the least significance difference (LSD) test. A probability value of < 0.05 was considered significant. All computations were made by employing SPSS statistical software (version 13.0).

3. Results

3.1. Antioxidant activity of ethanolic and aqueous extracts assessed by DPPH radical-scavenging method

DPPH is a free radical compound and has been used widely to test the free radical-scavenging ability of various samples. Antioxidants react with DPPH, reducing a number of DPPH molecules equal to the number of available hydroxyl groups. Therefore, the absorption at 517 nm was proportional to the amount of residual DPPH.

The results of the radical-scavenging capacity comparison between ethanolic extract, aqueous extract of Se-enriched green tea and α-tocopherol are shown in Table 1. The higher the inhibition rate, the greater the hydrogen-donating ability; thus the higher antioxidant activity. It is usual to calculate the inhibition percentage of antioxidant activity on DPPH at 30 min (Krings & Berger, 2001), although Yen and Duh (1994) measured the inhibition percentage at 16 min. In our studies, inhibition percentages were determined both at 15 and at 30 min. The ethanolic, aqueous extracts and α-tocopherol exhibited high antiradical activity with all inhibition rates exceeding 70%. The ethanolic extract manifested the highest inhibition rates (92.5% at 15 min and 92.7% at 30 min), whereas the aqueous extract showed the lowest inhibition (72.8% at 15 min and 78.0% at 30 min) at a concentration of 50 μg/ml. The antioxidant activity decreased at two time points following the same sequence: ethanolic extract > α-tocopherol > aqueous extract.

Table 1
Radical-scavenging capacity of ethanolic and aqueous extracts of Se-enriched green tea assessed by DPPH method

Samples	Inhibition rate[A] (%)	
	At 15 min	At 30 min
Ethanolic extract	92.5 ± 0.06[a]	92.7 ± 0.25[a]
Aqueous extract	72.8 ± 0.58[c]	78.0 ± 0.53[c]
α-tocopherol	85.2 ± 0.24[b]	88.5 ± 0.06[b]

Values are the means of three determinations ± SD. Values within a column followed by different letters are significantly different at $p < 0.05$.
[A] Inhibition rate was examined at a concentration of 50 μg/ml.

3.2. Antioxidant activity of ethanolic and aqueous extracts assessed by FTC method

The FTC assay was used to measure the amount of peroxide during the initial stages of lipid oxidation. A low absorbance is an indication of a low concentration of the formed peroxides and a high level of antioxidant activity. As shown in Fig. 1, both Se-enriched green tea extracts delayed the oxidation of linoleic acid, on the basis of low absorbance values, and exhibited higher antioxidant activity than control. In particular, the ethanolic extract of Se-enriched green tea showed a significantly stronger antioxidant activity than the aqueous extract and the positive control of α-tocopherol. The same patterns of activity were revealed as in the DPPH method: ethanolic extract > α-tocopherol > aqueous extract. Thus, the ethanolic extract of Se-enriched green tea had the highest inhibition activity of lipid oxidation. Altogether, these results suggested that the ethanolic extract of Se-enriched green tea possessed excellent antioxidant property.

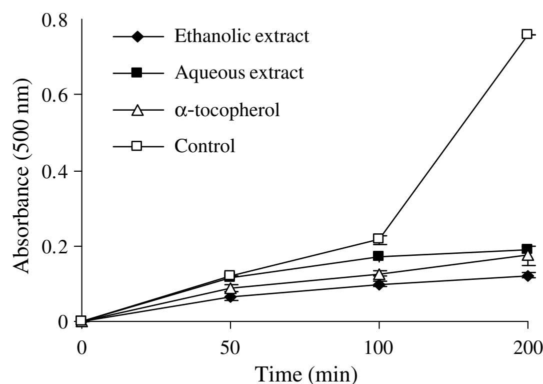

Fig. 1. Antioxidant activity of ethanolic and aqueous extracts of Se-enriched green tea, assessed by ferric thiocyanate (FTC) method at 50 μg/ml. Each value represents the mean ± SD of three determinations.

3.3. Evaluation of cytotoxic activity against human tumor cell lines

In our preliminary experiment, the anticancer activity of both extracts of Se-enriched green tea was evaluated against human cervical adenocarcinoma HeLa cell. As illustrated in Fig. 2, incubation with ethanolic extract and aqueous extract significantly inhibited the cells' proliferation in a dose-dependent manner, over the dose range of 15.625–250 μg/ml ($r_{ethanolic} = -0.748$, $r_{aqueous} = -0.960$, $p < 0.01$). At concentrations lower than 31.25 μg/ml, cell survival at the same dose followed the sequence: 5-FU < aqueous extract < ethanolic extract. However, over the dose range of 62.5–250 μg/ml, ethanolic extract showed significantly higher cytotoxicity against HeLa cells, compared with the aqueous extract and the positive control of 5-FU. Interestingly, the viability of cells treated with ethanolic extract sharply decreased, as the concentration varied from 31.25 to 125 μg/ml, and nearly reached 2%, even lower than 5-FU at 250 μg/ml. Altogether, the results revealed that the aqueous extract of Se-enriched green tea was inactive against HeLa cells, with an IC_{50} value higher than 300 μg/ml. In contrast, the ethanolic extract was found to be active against HeLa cells, with an IC_{50} of 90.0 μg/ml. Consequently, the result of the preliminary experiment prompted us to evaluate the antitumour activity of ethanolic extract against other cancer cells in the following study.

Further evidence for the *in vitro* inhibition of cancer cells by the ethanolic extract of Se-enriched green tea is provided by experiments with human lung carcinoma A549 and hepatoma HepG2 cell using MTT assay. As shown in Fig. 3, ethanolic extract significantly inhibited the proliferation of A549 (Fig. 3a) and HepG2 (Fig. 3b) in a concentration-dependent manner at 48 h, with IC_{50} values of 278 μg/ml and 432 μg/ml, respectively. Moreover, the maximum inhibitions of cell proliferation by ethanolic extract was observed at 600 μg/ml, with 25.4% survival for A549 and 22.4% survival for HepG2, respectively. All these data provided a strong evidence for the high anticancer effect of an ethanolic extract of Se-enriched green tea.

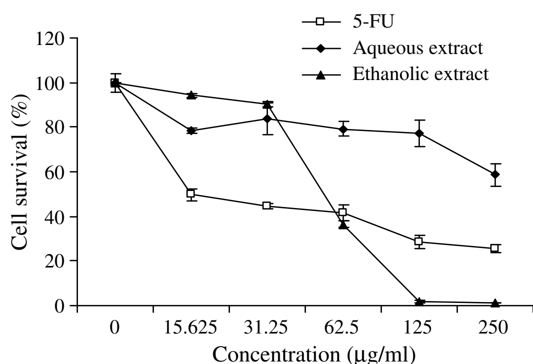

Fig. 2. Cytotoxic effect of ethanolic and aqueous extracts of Se-enriched green tea against human adenocarcinoma HeLa cell. Cell survival was determined as the percentage of the control from three independent experiments. Each value represents the mean ± SD of three determinations. 5-FU served as a positive control and exhibited an IC_{50} of 72.1 μg/ml.

3.4. Analysis of bioactive component in ethanolic extract and aqueous extract

The yields of ethanolic and aqueous extracts of Se-enriched green tea were 438 ± 3.43 mg/g and 309 ± 2.71 mg/g, respectively. The constituents analysis of ethanolic and aqueous extracts of Se-enriched green tea is shown in Table 2. Selenium and polyphenols content of the ethanolic extract of Se-enriched green tea was significantly higher than that of the aqueous extract. The protein content of the ethanolic extract of Se-enriched green tea was also significantly higher than that of the aqueous extract, which suggested the presence of abundant ethanol-soluble proteins in Se-enriched green tea. The polarity of 50% aqueous ethanol was between pure ethanol and water. Thus, both ethanol-soluble and aqueous protein could dissolve in the extraction solvent. However, the polysaccharide content of the ethanolic extract was significantly lower than the aqueous extract. This could be explained by the sedimentation effect of ethanol.

The results of the constituent analysis of tea polyphenols are shown in Table 3. The contents of EGC, DL-C, EC, EGCG and ECG in the ethanolic extract of Se-enriched green tea were significantly higher than those in the aqueous extract ($p < 0.05$). Interestingly, the content of EGCG in the ethanolic extract (276 mg/g) was more than 11-fold higher than that in the aqueous extract (24.0 mg/g). It is well known that polyphenols are better extracted in hydroalcoholic solution, which explained why there were significantly higher amounts of tea polyphenols and five individual catechins, especially EGCG, in the ethanolic extract of Se-enriched green tea. On the basis of the above analysis, it was concluded that the extraction solvent could significantly affect the content of bioactive components in the extract.

Table 2
Component analysis of ethanolic and aqueous extracts of Se-enriched green tea

Samples	Constituents			
	Selenium (μg/g)	Polyphenols (mg/g)	Protein (mg/g)	Polysaccharide (mg/g)
Ethanolic extract	4.35 ± 0.11[a]	558 ± 2.31[a]	258 ± 4.05[a]	134 ± 3.02[a]
Aqueous extract	4.03 ± 0.01[b]	383 ± 0.38[b]	64.5 ± 0.23[b]	188 ± 2.61[b]

Values are the means of three determinations ± SD. Values within a column followed by different letters are significantly different at $p < 0.05$.

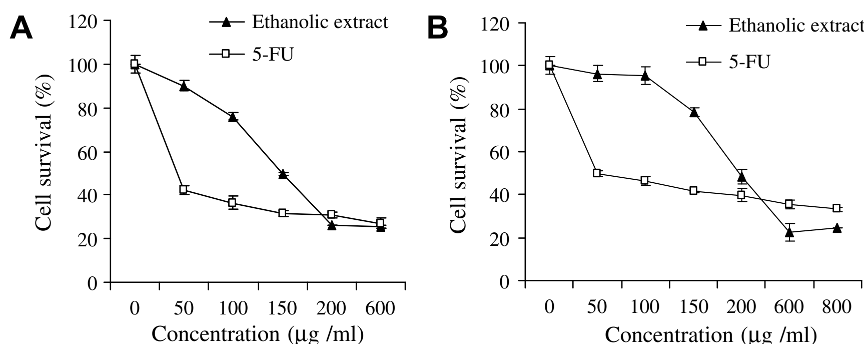

Fig. 3. Cytotoxic effect of ethanolic extract of Se-enriched green tea against human lung carcinoma cell A549 (A), and hepatoma cell HepG2 (B). Cell survival was determined as the percentage of the control from three independent experiments. Each value represents the mean ± SD of three determinations. 5-FU served as a positive control and exhibited an IC_{50} of 279 μg/ml for A549 and 258 μg/ml for HepG2.

Table 3
Constituent analysis of tea polyphenols of ethanolic and aqueous extracts of Se-enriched green tea

Components (mg/g)	Ethanolic extract	Aqueous extract
EGC	55.5 ± 0.14[a]	48.3 ± 0.07[b]
DL-C	10.5 ± 0.07[a]	1.90 ± 0.14[b]
EC	18.8 ± 0.14[a]	7.20 ± 0.14[b]
EGCG	276 ± 0.07[a]	24.0 ± 0.07[b]
ECG	52.6 ± 0.07[a]	3.60 ± 0.14[b]

EGC, epigallocatechin; DL-C, DL-catechin; EC, epicatechin; EGCG, epigallocatechin gallate; ECG, epicatechin gallate.
Values are the means of three determinations ± SD. Values within a row followed by different letters are significantly different at $p < 0.05$.

4. Discussion

Oxidation damage caused by free radicals can theoretically contribute to chronic diseases, such as cancer, cardiovascular disease, and age-related macular degeneration, and to aging (Seitz & Stickel, 2006). Selenium has been reported to be associated with antioxidant activity, anticancer effect and other physiological functions. Supplementation with inorganic selenium or various forms of organoselenium compounds in diets inhibited both chemically- and physically-induced oxidative damage (Letavayova et al., 2006). It has been proposed that selenium is a vital element of glutathione peroxidase, thioredoxin reductase and other selenium-containing enzymes, which protect against oxidation damage (El-Bayoumy & Sinha, 2005; Valko, Rhodes, Moncol, Izakovic, & Mazur, 2006). Accumulating evidence indicates that many selenoproteins, which contain selenium in the form of the amino acid selenocysteine, have important enzymatic functions associated with antioxidant activity (Gladyshev & Hatfield, 1999). Recently, a variety of studies showed an inverse relationship between the risk of certain types of cancer and the consumption of green tea, which is rich in tea polyphenols (Mohan, Gunasekaran, Varalakshmi, Hara, & Nagini, 2007; Thangapazham et al., 2007). Tea polyphenols have been well known as an effective scavenger of reactive oxygen species *in vitro* and *in vivo*. Numerous studies have demonstrated the anticarcinogenic activities of selenium and green tea polyphenols (Brinkman, Buntinx, Muls, & Zeegers, 2006; Thangapazham et al., 2007). Catechin, especially EGCG, was reported to be responsible for the biological activity of green tea, in particular antioxidant and anticancer activity (Hwang et al., 2007; Qin et al., 2007; Stewart, Mullen, & Crozier, 2005). Tea polyphenols are attracting attention as bioactive agents with anticancer, antidiabetic, antiviral, antimalarial, hepatoprotective, neuroprotective and cardioprotective effects (Adhami et al., 2007; Noonan, Benelli, & Albini, 2007).

Owing to the traditional consumption of green tea infused with hot water, most studies have focused on the water extraction of green tea. However, the contents of active components in the extract of green tea vary with different extraction methods. The extraction yield of bioactive components depends not only on the time and temperature of extraction, but also predominantly on the extraction solvent (Bazinet, Labbe, & Tremblay, 2007; Turkmen et al., 2006). Consequently, an optimal extraction for the constituents of Se-enriched green tea using 50% (v/v) aqueous ethanol was conducted.

Due to the complex nature of extracts derived from Se-enriched green tea, it is insufficient to evaluate the antioxidant activity of extracts only employing a single method. Therefore, the DPPH radical-scavenging assay and the FTC method were performed, to determine the abilities of the extracts to inhibit oxidation (Table 1 and Fig. 1). The ethanolic extract of Se-enriched tea exhibited the strongest antioxidant activity. A similar result has been reported by Turkmen et al. (2006), who investigated the effect of different extracting solvents on the antioxidant activity of black and black mate tea extracts, and found that an ethanolic extract prepared with 50% ethanol (v/v) exhibited considerably higher antioxidant capacity, compared with an aqueous extract (Turkmen et al., 2006). In our previous study, the Se-enriched green tea with a lower content of TPP possessed higher antioxidant activity than the regular tea (Xu, Yang, et al., 2003). Therefore, this implied that the contribution to the activity was not only from the higher level of TPP (Table 2), but also from selenium in enhancing the antioxidant activity of ethanol extracts of Se-enriched tea.

Cervical adenocarcinoma, lung carcinoma and hepatoma are the most frequent causes of cancer death and are the commonly diagnosed cancers in humans worldwide. Therefore, the cell growth inhibition of ethanolic and aqueous extracts of Se-enriched tea was evaluated using *in vitro* cell models of human cervical adenocarcinoma HeLa, lung carcinoma A549 and hepatoma HepG2 cells. Ethanolic extracts manifested significantly higher growth inhibition than aqueous extracts and a positive control toward Hela cells, which indicated that the extraction of bioactive substances from Se-enriched tea with ethanol was more efficient than the extraction with hot water (Fig. 2). The data from Fig. 3 further confirmed high anticancer activity of ethanolic extracts, especially at higher concentrations. To well understand the relationship between the bioactive substances of both extracts and high antioxidant and anticancer capacity, component analysis was conducted; the results revealed a higher content of selenium, tea polyphenol (especially EGCG) and protein in the ethanolic extract (Tables 2 and 3). This result was in agreement with Perva-Uzunalic's reports that the extraction efficiency and the overall content of major catechins obtained by 50% ethanol (v/v) were superior to that obtained by water (Perva-Uzunalic et al., 2006). In addition, the results of another study in our laboratory also showed that the ethanolic extract of Se-enriched tea possessed higher antitumour activity against human lung cancer A549 cells, compared with that of the regular tea, as well as the combination of the extract of regular green tea and sodium selenite, which contains the same amounts of selenium as Se-enriched tea (data not shown). All these results suggested that the enhancement of antioxidant and anticancer activity might not only be attributed to the presence of a higher content of selenium and tea polyphenol in the ethanolic extract, but also in part due to selenium in combination with phenolic or nonphenolic compounds. However, further studies are needed to clarify if selenium was acting in combination with phenolic or nonphenolic compounds, as well as the determination of the possible structure of the components in ethanolic extracts of selenium-enriched tea responsible for high antioxidant and anticancer activity.

5-FU has been one of the most widely used anticancer agents for over 40 years and known to affect the cell cycle and to induce apoptotic death of cancer cells (Li et al., 2004). According to clinical observation, however, severe toxicity of 5-FU was found in some cancer patients (Van Kuilenburg, 2004). Thus, a biologically active extract of Se-enriched tea could be accepted as a chemopreventive agent and developed for application in the prevention and treatment of cancer. A key issue in the cancer chemopreventive nature of the extract is whether the activity and molecular mechanism of the extract is due to a single compound. However, the active extract may be preferable to single compounds for two reasons: (1) it is very expensive to identify and develop isolated compounds and (2) a tea extract with various active compounds could be gentler than a pure active compound.

5. Conclusion

In summary, to our knowledge, this is the first time that the antioxidant and anticancer activities of ethanol extract of Se-enriched green tea have been evaluated. The results indicated that the ethanol extract of Se-enriched green tea possessed significantly

higher antioxidant activity than the aqueous extract, by both DPPH radical-scavenging and FTC methods. Furthermore, an ethanolic extract of Se-enriched green tea, containing significant amounts of EGCG and selenium, also inhibited the growth of HeLa, A549 and HepG2 cancer cells *in vitro*.

Se-enriched tea extract could be potentially used in food as a natural antioxidant, to take the place of synthetic antioxidants. Although Se-enriched green tea and its active constituents have been shown to inhibit cancers, the exact mechanisms of cancer prevention are not clearly understood. Thus, further studies are needed, for (1) the elucidation of the interaction of the components in Se-enriched green tea and their synergistic effects on enhancing antioxidant and antitumour activity and (2) the observation of *in vivo* biological effects of extracts derived from Se-enriched green tea, using an animal model.

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