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

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ABSTRACT

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.

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