JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Compositional Analysis and Preliminary Toxicological Evaluation of a Tea Polysaccharide Conjugate

HAIXIA CHEN,*,† MIN ZHANG,‡ ZHISHUANG QU,† AND BIJUN XIE§

School of Pharmaceutical Science and Technology, Tianjin University, Tianjin, 300072, People's Republic of China, Faculty of Food Engineering and Biotechnology, Tianjin University of Science & Technology, Tianjin 300222, People's Republic of China, and College of Food Science and Technology, Huazhong Agricultural University, Wuhan, 430070, People's Republic of China

Tea polysaccharide conjugate is one of the main bioactive constituents of tea (*Camellia sinensis* L.). The chemical composition and preliminary toxicological evaluation of a tea polysaccharide conjugate was investigated to determine the possibility of using it for human consumption. Chemical analysis of tea polysaccharide conjugate showed that the tea polysaccharide conjugate was a nonstarch protein bounded acidic polysaccharide. The protein, neutral sugar, and uronic acid content of the tea polysaccharide conjugate was 3.5%, 44.2%, and 43.1%, respectively. The contents of iron, magnesium, zinc, and selenium in the tea and its polysaccharide conjugate were measured by inductively coupled plasma-optical emission spectrometry (ICP-OES). Results showed that the contents of four elements in tea polysaccharide conjugate were much higher than that of tea power. Especially, the content of iron in tea polysaccharide conjugate was increased 5.9 times. The tea polysaccharide conjugate, when fed to mice, was found to have no toxicity to the liver, kidney, heart, thymus, or spleen of the mice and none of the mice died throughout the period of the experiment. There was no significant difference between the thymus index, spleen index, and liver index of the mice from the test and control groups ($P > 0.05$). On the basis of the study, the tea polysaccharide conjugate may be classified either as a very low toxicity substance, that is, GHS Category 5 (globally harmonized system), or as unclassified when orally administrated to mice. It might be a candidate of dietary supplements besides the bioactivities as a polysaccharide.

KEYWORDS: Tea polysaccharide conjugate; composition; toxicological evaluation

INTRODUCTION

Tea (*Camellia sinensis* L.) has been used as a beverage in China and Japan for thousands of years. It is the second most consumed beverage in the world next to water. The tea plant has been widely used for centuries by ancient cultures for its medicinal properties and is popularly consumed in unfermented (green tea), semifermented (oolong teas), and fermented (black and pu-erh or red) forms (*1*). Traditionally, tea was drunk to improve blood flow, to eliminate toxins, and to improve resistance to diseases (*2*, *3*). Now, it is found that tea could protect against chemically induced tumor initiation and promotion and progression of benign tumors to malignancy (*4*). Furthermore, the medicinal activities of tea include antioxidant (*5*), antiatherosclerosis (*6*), anti-hypertension (*7*), anti-infectious diseases (*8*), and improving immune response (*9*). Folk remedies have also included the antidiabetic properties of tea for decades (*10*). The chemical composition of tea includes proteins, polysaccharides, chlorophyll, minerals and trace elements, volatile compounds, amino and organic acids, lignins, alkaloids (caffeine, theophylline, and theobromine), polyphenols (catechins or flavan-3-ols, theaflavins, thearubigins, and proanthocyanidins) and so forth (*11*).

The human body requires many minerals to maintain good health. For instance, calcium is needed for bone growth and muscle contraction and in blood clotting (*12*). Magnesium works with calcium to maintain healthy bones (*13*). Selenium is an essential nutrient for humans, and it is especially important for indigenous antioxidative defense system and immunological defense (*14*, *15*). Recently, selenium also was found to be a cancer-protective agent with its function of preventing and counteracting cancer (*16*−*18*). Zinc is also an essential trace element for virtually all-living species (*19*). Zinc is an important constituent of dozens of metalloenzymes and metalloproteins, and it plays an essential role in several biological processes involved in normal growth and development (*20*). Many minerals essential to human nutrition accumulate in different parts of plants. Many plants act as a natural source of concentrated mineral nutritional supplements (*21*).

* To whom correspondence should be addressed. Phone: 86-22-27401483. Fax: 86-22-27892025. E-mail: chennhxx@yahoo.com.cn.
† School of Pharmaceutical Science and Technology, Tianjin University.
‡ Faculty of Food Engineering and Biotechnology, Tianjin University of Science & Technology.
§ Huazhong Agricultural University.

10.1021/jf0632740 CCC: $37.00
Published on Web 02/17/2007

The consumption of green tea is especially popular in Asian countries, and its association with human health benefits has resulted in the inclusion of green tea extracts as common botanical ingredients in dietary supplements, nutraceuticals, and functional foods (*22*). Tea polysaccharide conjugate is one of the main bioactive components of green tea, especially the low-grade green tea. The content decreases with the quality grade improvement of the tea. The content of tea polysaccharide conjugate was from 0.8% to 1.5% in low-grade tea and from 0.4% to 0.9% in high-grade tea according to Wang et al.'s study (*23*). Tea polysaccharide conjugate is one of the main components in tea related to hypoglycemic activity according to prior studies (*24*, *25*). Therefore, low-grade tea has traditionally been used to cure diabetes in East Asia, especially in China and Japan. Great advances have been made in chemical and bioactive studies of tea polysaccharide conjugate in recent decades. It is identified as an acidic polysaccharide with protein and mineral elements conjugated (*26*−*28*). The monosaccharide constituents and molecular weight of tea polysaccharide conjugate were not the same depending on the type of tea and isolation method. Tea polysaccharide conjugate was reported to have immunological, antiradiation, antiblood coagulation, anticancer, anti-HIV, and hypoglycemic activities (*29*, *30*). However, there is no information on mineral element content and toxicological evaluation of tea polysaccharide conjugate.

The objectives of this study were to analyze the composition including the mineral elements and to evaluate the preliminary acute toxicity of tea polysaccharide conjugate. This will be helpful for determining the possibility for human consumption and exploring the new function of food products with tea polysaccharide conjugate.

MATERIALS AND METHODS

Materials. Low-grade green tea leaf (*Camellia sinensis*) was purchased from Xuanen County Tea Factory, Hubei Province, China. It was identified as six-grade crude green tea according to the Green Tea quality standard (GH016-84) of China. Trifuoroacetic acid (TFA) and the standard monosaccharides (D-glucose, D-xylose, D-galactose, D-ribose, L-arabinose, D-galacturonic acid) were purchased from Merck Co. (Darmstadt, Germany) and Sigma Chemical Co. (St. Louis, MO). Standard solutions of the four elements including iron, magnesium, zinc, and selenium were prepared immediately by dilutions of a 1000 mg/L stock solutions (Merck, Darmstadt, Germany) prior to use. The dilutions of the four elements were at the same concentrations of 0.1, 1, 10, 100, and 1000 mg/L for each calibration curve. Milli-Q deionized water (Millipore, Milford, MA) was used throughout this experiment. All solvents and reagents such as HNO_3, HCl, and H_2O_2 were of analytical reagent grade (Merck). All glassware and equipment were soaked with 10% HNO_3 at least overnight and then were rinsed with deionized water prior to use.

Sample Preparation. Low-grade green tea leaf was smashed to powers. Tea polysaccharide conjugate was extracted from the tea powders, and impurities were excluded by the procedures as our previously described method (*27*). Briefly, the isolation procedure was as follows. Low-grade green tea powders (100 g) were mixed with 500 mL of 80% (v/v) ethanol and were shaken at 30 °C for 24 h to remove most of the polyphenols and monosaccharide. After the mixture was filtered, the residues were dried in air and then were extracted with hot water (70 °C) three times (1:20, w/v), 30 min each time. The extracted solution was concentrated to 10% of the original volume with a rotary evaporator under reduced pressure, and then it was precipitated by adding four times of volume of 95% (v/v) ethanol at 4 °C for 24 h. The sediment was centrifuged (5000*g*, 10 min) and vacuum freeze-dried, and 2.9 g of crude tea polysaccharide conjugates was obtained. Crude tea polysaccharide conjugates were then purified by an adsorption column (60 cm × 3.0 cm, i.d.) packed with polyamide resin (Nankai University Chemical Co., Tianjin, China). Polyamide was a polymer of hexanolactam to remove polyphenolic compounds. Briefly, the adsorption column was primed by washing with five column volumes of 0.5 M NaOH, followed by five column volumes of 0.5 M HCl, and 95% (v/v) ethanol was applied last. Before changing the wash solutions, 20 column volumes of distilled water were applied to wash the adsorption column. The adsorption column was then equilibrated with destilled water. The 2.9 g of crude tea polysaccharide conjugate (dissolved in 0.5 mL distilled water) was applied to the column and was then eluted with distilled water at a flow rate of 0.4 mL/min. The amount of glycan per fraction was detected using the sulfuric acid−phenol method (*31*), and the protein eluted was detected automatically by monitoring absorbance at 280 nm (*32*). The glycan fractions were collected and precipitated with four times of volume of 95% (v/v) ethanol and then were lyophilized. Purified tea polysaccharide conjugate was obtained, and the yield was about 1.0%.

Physicochemical Analysis. The physicochemical properties of the tea polysaccharide conjugate were charactered by solubility detection and colorimetric assays including iodine assay, Folin−Ciocalteu reagent reaction, carbazole assay, and Coumassie-blue assay (*33*−*36*).

The carbohydrate content of the green tea power and tea polysaccharide was determined by the phenol−sulfuric acid method (*31*). In brief, 2 mL of sample solution was vortex mixed with 1 mL of 5% phenol in water before adding 5 mL of concentrated sulfuric acid rapidly from a glass dispenser. After standing for 20 min at room temperature, the absorbance of the sample solution was measured at 490 nm against the blank (prepared by substituting distilled water for the sample solution). The amount of total carbohydrates was determined by reference to a standard curve made from glucose. The concentrations of glucose for standard were 160, 80, 40, 20, 10, and 5 μg/mL. Analysis of monosaccharide constituents of tea polysaccharide conjugate was performed with a Shimadzu GC-6A (Japan) gas chromatography (30 m × 0.32 mm i.d. WCOT column containing OV1701) according to the method of Chaplin and Kennedy (*35*).

The uronic acid content of tea polysaccharide conjugate was quantified by high-performance liquid chromatography (HPLC) on a Sugar-Pak I column with a 1.0×10^{-4} mol/L calcium disodium ethylenediaminetetraacetic acid solution as the mobile phase and refractive index detection as described by Chen et al. (*37*). The protein content in tea power and tea polysaccharide conjugate was measured according to Coumassie-blue assay using bovine serum albumin (BSA) as the standard (*36*). Assay of polyphenolic compounds in tea power and tea polysaccharide was conducted according to Bonvehi and Coll's method (*34*).

Mineralization of the Samples. The wet-ashing method was employed for the digestion of the sample according to Ajayi et al. (*38*). An amount of 0.1000 g of the dry tea polysaccharide conjugate and green tea samples was digested with 10 mL of concentrated HNO_3 and perchloric acid (1:1 v/v) and thereafter was transferred to a 25-mL volumetric flask. It was diluted to volume with deionized water and was stored in a clean polyethylene bottle. The mineral element content was determined by an inductively coupled plasma optical emission spectrometer (ICP-OES).

Analysis of Mineral Elements. Elements were determined using an ICP-OES (Varian, VISTA-MPX, United States) equipped with an axial torch, Scott-type spray chamber, and cross-flow nebulizer with gemtips. The plasma conditions are as follows: RF power 1000 W, nebulizer flow 0.5 L/min, auxiliary flow 1.0 L/min, plasma flow 15 L/min, and sample flow 1.5 mL/min. The wavelength used in the instrument and method detection limits for elements iron, magnesium, zinc, and selenium were 273.61, 213.86, 279.56, 196.04 nm and 0.003, 0.02, 0.002, 0.003 μg/mL, respectively. An autosampler was used for the introduction of the solutions into the nebulizer. Standards, analytical blanks, and rinse blanks were matrix-matched to the sample so that all solutions contained 20% concentrated HNO_3 by volume.

Acute Toxicity Study of Tea Polysaccharide Conjugate. Kunming male mice (4-week-old mice, weighing between 18 and 22 g) were provided by the Animal Center, Institute of Health and Epidemic Prevention (Wuhan, China). The mice were housed under normal laboratory conditions with free access to standard rodent chow (supplied by the Animal Center, Institute of Health and Epidemic Prevention, Wuhan, China) and water in the acclimation period. Twenty-two mice

Table 1. Mineral Element Contents of Tea Polysaccharide Conjugate and Tea

element	tea power (mg/ kg)	tea polysaccharide conjugate (mg/kg)	ratio[a]
iron	404.8	2389.1	5.9
zinc	52.7	241.9	4.6
magnesium	1842.5	4643.6	2.5
selenium	1.0	3.6	3.6

[a] Ratio is expressed by element contents of tea polysaccharide conjugate relative to that of tea power.

were separated into two groups randomly. At the commencement of the experiment, test group mice were orally administrated a total of 5.0 g kg^{-1} body weight (b. wt) of the polyamide column purified tea polysaccharide conjugate (dissolved in physiological saline solution, 0.5 mL at a dose, two doses in 24 h) in the first day, while the control group received the same volume of physiological saline solution alone. Then, the animals had free access to food and water throughout the experimental period. The body weight of each mouse was recorded every day for the 15 days of the experiment. Animals were sacrificed after a 14−16 h overnight fast on the last day of the experiment. For reasons of low toxicity of polysaccharide concern and as regulations recommend (OECD Test Guideline 420; fixed dose procedure), testing on animals in GHS Category 5 ranges (5.0 g/kg) was chosen in this experiment.

Tissue Examination. The abdominal wall was dissected through the linear alba and peritoneum using a scalped blade. The thymus and liver of each mouse were examined for gross lesions, and thymus index, spleen index, and liver index were determined. Thymus index, spleen index, and liver index were expressed as the thymus, spleen, and liver weight relative to body weight, respectively (mg/g).

Statistics. All the data were expressed as means ± standard deviation (SD) of three replications, and Student's test was used for the statistical analysis. The values were considered to be significantly different when the P value was less than 0.05.

RESULTS

Chemical Composition of the Tea Polysaccharide Conjugate. The tea polysaccharide conjugate was soluble in water but was not soluble in organic solvent such as ethanol, aether, acetone, and chloroform. There were no reactions of Folin−Ciocalteu reagent with tea polysaccharide conjugate, which suggested that no polyphenols existed in the tea polysaccharide conjugate. Results of carbazole assay, Coumassie-blue assay, and iodine assay suggested that the tea polysaccharide conjugate was a nonstarch protein bounded acidic polysaccharide.

The protein content, neutral sugar content, and polyphenol content of tea powder were 17.2%, 4.2%, and 26.2%, respectively. Compared with tea power, no polyphenols existed in the tea polysaccharide conjugate, and the content of protein in the tea polysaccharide conjugate was reduced to 3.5%. This may be because the free protein was removed during the isolation procedure of polysaccharide. The content of neutral sugar was 44.2% and it was composed of Ara, Rib, Xyl, Glc, and Gal in mole ratios of 1.0:0.8:2.7:0.9:0.4. The uronic acid that existed in tea polysaccharide conjugate was identified as galacturonic acid by HPLC, and the content was 43.1%.

Mineral and Trace Elements. Element contents of iron, magnesium, zinc, and selenium were determined by ICP-OES. The mineral element contents of tea polysaccharide conjugate and tea are listed in **Table 1**. Significantly higher contents of the four elements were found in the tea polysaccharide conjugate than in the tea power. The results showed that the tea had a high level of magnesium, 1842.5 mg/kg, followed by iron, 404.8 mg/kg, zinc, 52.7 mg/kg, and a low level of selenium, 1.0 mg/kg. The content ratio ranged from 2.5 to 5.9. Especially, the content of iron of tea polysaccharide conjugate was 2389.1 mg/kg, which was 5.9 times of that of tea power. Apparently, the elements were enriched during the isolation procedures of tea polysaccharide conjugate.

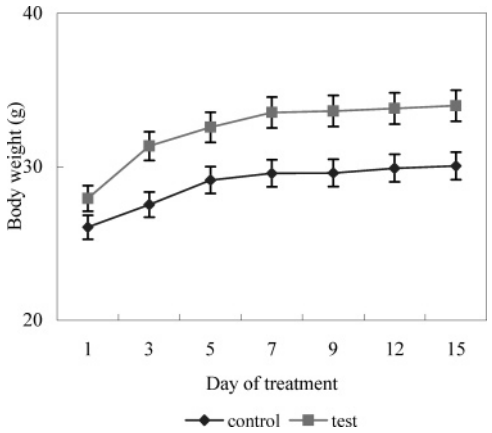

Figure 1. Effect of tea polysaccharide conjugate on body weight gain of mice by oral administration ($n = 11$).

Effect of Tea Polysaccharide Conjugate on Growth of Mice. Oral administration of two doses of the tea polysaccharide given within 24 h was utilized and mice were observed through a 15-day period. The body weight gain of the test and control mice during the experiment period is shown in **Figure 1**. Both the control and test group mice grew well. There were no significant alterations between the body weight gain of the mice from the test and control groups ($P > 0.05$). Furthermore, mice from the test group displayed a little higher body weight gain compared to those from the normal control group. The mice of the two groups were observed to be healthy and active. No mortality was recorded in any of the control and test mice throughout the duration of study.

Tissue Examination. No lesions were observed in the liver, kidney, thymus, spleen, or heart of mice that received 5.0 g/kg. Seen from **Table 2**, there was no significant difference between the thymus index, spleen index, and liver index of the mice from the test and control groups ($P > 0.05$). Furthermore, the spleen index and the thymus index of the test group were increased slightly. No gross abnormalities of toxicological significance were noted for any animals during necropsy examination. Hence, LD_{50} of tea polysaccharide conjugate is >5.0 g/kg b.wt. The absence of toxicity at a concentration as high as 5.0 g/kg b.wt. means that the substance may be classified either as a very low toxicity substance, that is, GHS Category 5 (globally harmonized system), or as unclassified. Considering safety factors of 100 from mice to humans, 500 mg/kg b.wt. might be extrapolated as a very low toxicity dose for humans from the obtained results.

DISCUSSION

In recent decades, it has been found that polysaccharides are not only energy resources but also play key biological roles in many life processes as well. Cell-surface carbohydrates are major components of the outer surface of mammalian cells and are very often characteristics of cell types. It is assumed that cell type-specific carbohydrates are probably involved in cell−cell interaction, in particular, as molecules that are recognized by carbohydrate-binding proteins. Structure of oligosaccharide of cell-surface glycoconjugate is correlated with vast physiologically important functions, such as cell recognition, cell

Table 2. Effect of Tea Polysaccharide Conjugate on Tissue of Mice by Oral Administration[a]

group	liver weight (mg)	spleen weight (mg)	thymus weight (mg)	liver index (mg)	spleen index (mg/g)	thymus index (mg/g)
control	1232.4 ± 101.7	78.9 ± 39.0	65.2 ± 32.6	42.4 ± 9.0	2.5 ± 0.9	2.1 ± 0.9
test	1293.7 ± 120.1	92.9 ± 25.8	91.1 ± 27.2	38.7 ± 7.5	2.7 ± 0.7	2.6 ± 0.5

[a] Values are means ± standard deviation, $n = 11$.

growth, contact inhibition, and cell differentiation (*39*−*41*). Natural polysaccharides do not always exist in plants, animals, and microorganisms singly but conjugate with other components including protein, lipids, nuclear acids, and so forth. During the isolation procedure of polysaccharides, the nonsaccharide components are always removed by chemical or enzyme methods. Sometimes, the polysaccharide conjugates act as one homogeneous compound. In this study, we obtained a tea polysaccharide conjugate, which contained neutral sugar, galacturonic acid, protein, and mineral elements according to the chemical analysis and mineral analysis.

Many people use some methods of complementary and alternative medicine therapy including diet, sport, sunshine bath, and so forth. The use of dietary supplements (botanicals or herbals, vitamins, and minerals) is extremely popular. Minerals and trace elements are rich in tea. In this study, inductively coupled plasma atomic emission spectrometry (ICP) was used for the comparative analysis of the minerals and trace elements of the tea power and tea polysaccharide conjugate. It was found that there was an enrichment of the minerals and trace elements in tea polysaccharide conjugate. This result indicated the possibility that tea polysaccharide conjugate could act as a candidate for the mineral supplement. Compared with previous studies (*42*), our results showed the low content of elements in low-grade green tea power as compared to the high-grade green tea power. There were studies on the composition of tea polysaccharide conjugates in which mineral elements existed (*43*). However, no information was available regarding the content changes of the mineral elements in tea power and tea polysaccharide conjugate. Furthermore, compared with tea power or tea extract, tea polysaccharide conjugate is a relatively homogeneous compound having the bioactivities of polysaccharides besides the mineral supplement function. The low-grade tea is usually unmarketable. It is applicable to produce tea polysaccharide conjugate because of the high content. The new finding of the mineral element enrichment of tea polysaccharide conjugate may benefit the utilization of low-grade tea. The preparation procedure of using polyamide column purification is fit to be magnified for industry produce. Thus, it might be easy to produce tea polysaccharide conjugate commercially.

Tea polysaccharide conjugate was found to have multiple bioactivities including immunological, antiradiation, antiblood coagulation, anticancer, anti-HIV, and hypoglycemic activities in prior studies (*27*−*30*). The preliminary toxic effect of tea polysaccharide conjugate was evaluated in this study. It was found that there were no lesions observed in the organs and tissues of mice at 5.0 g/kg b.wt. level by oral administration. However, the dose−response experiment for the acute toxicity, subtoxicity, and chronic toxicity will need further studies.

In conclusion, the physicochemical properties of tea polysaccharide conjugate showed that it was a protein bounded acidic polysaccharide coupled with mineral elements. The content of the mineral elements in tea polysaccharide conjugate was much higher than that of the original green tea power. Gross observation did not show toxic effects to the liver, kidney, heart, thymus, or spleen of the mice and none of the mice died throughout the period of the experiment. The tea polysaccharide conjugate may be classified either as a very low toxicity substance, that is, GHS Category 5 (globally harmonized system), or as unclassified. The tea polysaccharide conjugate might be a candidate for dietary supplements besides the bioactivities as a polysaccharide. Subacute and chronic toxicological studies in animals are also needed before clinical trials.

LITERATURE CITED

(1) Zhu, Q. Y.; Hackman, R. M.; Ensunsa, J. L.; Holt, R. R.; Keen, C. L. Antioxidative Activities of Oolong Tea. *J. Agric. Food Chem.* **2002**, *50*, 6929−6934.

(2) Weisburger, J. H. Tea and health: ahistorial perspective. *Cancer Lett.* **1997**, *114*, 315−317.

(3) Hara, Y. *Green Tea, Health Benefits and Applications*; Dekker: New York, 2001.

(4) Mukhtar, H.; Katiyar, S. K.; Agarwal, R. Green tea and skins anticarcinogenic effects. *J. Invest. Dermatol.* **1994**, *102*, 3−7.

(5) Yen, G. C.; Chen, H. Y.; Peng, H. H. Antioxidant and pro-oxidant effects of various tea extracts. *J. Agric. Food. Chem.* **1997**, *45*, 30−34.

(6) Curin, Y.; Andriantsitohaina, R. Polyphenols as potential therapeutical agents against cardiovascular diseases. *Pharmacol. Rep.* **2005**, *57*, 97−107.

(7) Hodgson, J. M.; Burke, V.; Puddey, I. B. Acute effects of tea on fasting and postprandial vascular function and blood pressure in humans. *J. Hypertens.* **2005**, *23*, 47−54.

(8) Weber, J. M.; Ruzindana-Umunyana, A.; Imbeault, L.; Sircar, S. Inhibition of adenovirus infection and adenain by green tea catechins. *Antiviral Res.* **2003**, *58*, 167−173.

(9) Bhattaxharyya, A.; Mandal, D.; Lahiry, L.; Sa, G.; Das, T. Black tea protects immunocytes from tumor-induced apoptosis by changing Bcl-2/Bax ratio. *Cancer Lett.* **2004**, *209*, 147−154.

(10) Anderson, R. A.; Polansky, M. M. Tea enhances insulin activity. *J. Agric. Food Chem.* **2002**, *50*, 7182−7186.

(11) Seeram, N. P.; Henning, S. M.; Niu, Y.; Lee, R.; Scheuller, H. S.; Heber, D. Catechin and caffeine content of green tea dietary supplements and correlation with antioxidant capacity. *J. Agric. Food Chem.* **2006**, *54*, 1599−1603.

(12) Bryant, R. J.; Cadogan, J.; Weaver, C. M. The new dietary reference intakes for calcium: implications for osteoporosis. *J. Am. Coll. Nutr.* **1999**, *18*, 406S−412S.

(13) Matsuzaki, H. Prevention of osteoporosis by foods and dietary supplements. Magnesium and bone metabolism. *Clin. Calcium* **2006**, *16*, 59−64.

(14) de Lorgeril, M; Salen, P. Selenium and antioxidant defenses as major mediators in the development of chronic heart failure. *Heart Failure Rev.* **2006**, *11*,13−17.

(15) Duntas, L. H. The role of selenium in thyroid autoimmunity and cancer. *Thyroid* **2006**, *16*, 455−460.

(16) He, P.P.; Lv, X.Z.; Wang, G.Y. Effects of Se and Zn supplementation on the antagonism against Pb and Cd in vegetables. *Environ. Int.* **2004**, *30*, 167− 172.

(17) Gavin, E.A.; Helmut, S. The biochemistry of selenium and the glutathione system. *Environ. Toxicol. Pharmacol.* **2001**, *10*, 153− 158.

(18) Korpela, H. Selenium in cardiovascular diseases. *J. Trace Elem. Electrolytes Health Dis.* **1993**, *7*, 115.

(19) Frassinetti, S.; Bronzetti, G.; Caltavuturo, L.; Cini, M; Croce, C. D. The role of zinc in life: a review. *J. Environ. Pathol. Toxicol. Oncol.* **2006**, *25*, 597−610.

(20) Kasprzak, K. S.; Waalkes, M. P. The role of calcium, magnesium, and zinc in carcinogenesis. *Adv. Exp. Med. Biol.* **1986**, *206*, 497−515.
(21) Mark, P. E.; Michael, J. B.; Jianwei, W. H.; Christopher, D. G. Plants as a natural source of concentrated mineral nutritional supplements. *Food Chem.* **2000**, *71*, 181−188.
(22) Nookabkaew, S.; Rangkadilok, N.; Satayavivad, J. Determination of Trace Elements in Herbal Tea Products and Their Infusions Consumed in Thailand. *J. Agric. Food Chem.* **2006**, *54*, 6939−6944.
(23) Wang, D. F.; Xie, X. F.; Cai, C.Y.; Yang, M. Analysis of Pharmacological components of coarse tea curing diabetes. *Chin. Tradit. Herb. Drugs* **1995**, *26*, 255−257.
(24) Chen, H. X.; Xie, B. J. The preventive and curative effect on diabetic mice of tea polysaccharides. *Acta Nutr. Sin.* **2002**, *24*, 85−86.
(25) Ding, R. F.; He, P. M.; Jie, G. L. Study on the hypoglycemic mechanism of tea polysaccharides and tea polyphenols. *J. Tea Sci.* **2005**, *25*, 219−224.
(26) Tadakazu, T.; Tomoki, U.; Hitoshi, K.; Fumihisa, Y.; Mistugu, M. The chemical properties and functional effects of polysaccharides dissolved in green tea infusion. *Nippon Shokuhin Kagaku Kogaku Kaishi* **1998**, *45*, 270−272.
(27) Chen, H. X.; Zhang, M.; Xie, B. J. Components and antioxidant activity of polysaccharide conjugate from Green Tea. *Food Chem.* **2005**, *90*, 17−21.
(28) Chen, H. X.; Zhang, M.; Xie, B. J. Spectroscopy investigation on conformational transition of tea glycoconjugate from green tea. *Chin. J. Chem.* **2004**, *22*, 1387−1390.
(29) Chen, H. X.; Xie, B. J. Progress on the pharmacological activities of tea polysaccharides. *J. Chin. Med. Mater.* **2001,** *24*, 65−67.
(30) Zhou, J.; Ding, J. P.; Wang, Z. N.; Xie, X. F. Effect of tea polysaccharides on blood-glucose, blood lipid and immunological function of mice. *J. Tea Sci.* **1997**, *17*, 75−79.
(31) Dubois, M.; Gilles, K. A.; Hamilton, J. K.; Rebers, P. A.; Smith, F. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* **1956**, *28*, 350−356.
(32) Chinpal, K.; Frederick, L. C.; Page, W. F. D.; James, M. Purification and characterization of a doxorubicin-inhibited NADH-quinone (NADH-ferricyanide) reductase from rat liver plasma membranes. *J. Biol. Chem.* **2002**, *277*, 16441−16447.
(33) Rendleman, J. R.; Jacob, A. The reaction of starch with iodine vapor. Determination of iodide-ion content of starch−iodine complexes. *Carbohydr. Polym.* **2003**, *51*, 191−202.
(34) Bonvehi, J. S.; Coll, F. V. Evaluation of bitterness and astringency of polyphenolic compounds in cocoa powder. *Food Chem.* **1997**, *60*, 365−370.
(35) Chaplin, M. F.; Kennedy, J. F. *Carbohydrate analysis: A practical approach;* Oxford University Press: Oxford, 1994.
(36) Bradford, M. M. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein binding. *Anal. Biochem.* **1976**, *72*, 248−254.
(37) Chen, H. X.; Zhang, M.; Xie, B. J. Quantification of uronic acids in tea polysaccharide conjugates and its antioxidant properties. *J. Agric. Food. Chem.* **2004**, *52*, 3333−3336.
(38) Ajayi, I. A.; Oderinde, R. A.; Ogunkoya B. O.; Egunyomi, A.; Taiwo, V. O. Chemical analysis and preliminary toxicological evaluation of Garcinia mangostana seeds and seed oil. *Food Chem.* **2007**, *101*, 999−1004.
(39) Bucior, I.; Burger, M. M. Carbohydrate-carbohydrate interactions in cell recognition. *Curr. Opin. Struct. Biol.* **2004**, *14*, 631−637.
(40) Ari, H.; Markus, A. Intracellular functions of N-Linked Glycans. *Science* **2001**, *291*, 2364−2369.
(41) Lu, Y.; Pelling, J. C.; Chaney, W. Tumor cell surface beta 1-6. branched oligosaccharides and lung metastasis. *Clin. Exp. Metastasis* **1994**, *12*, 47−54.
(42) Gallaher, R. N.; Gallaherb, K.; Marshalla, A. J.; Marshall, A. C. Mineral analysis of ten types of commercially available tea. *J. Food Compos. Anal.* **2006**, *19*, S53−S57.
(43) Wang, D. F.; Li, J.; Wang, C. H.; Zhao, G. W.; Jin, Y.; Cheng, D. D.; Ye, S. Study on the component and immune activity of polysaccharides from tea. *J. Tea Sci.* **2000**, *20*, 45−50.

Received for review November 13, 2006. Revised manuscript received January 5, 2007. Accepted January 16, 2007. We are grateful for the financial support of this research from the National Natural Science Foundation of China (Grant No. 30600470) and the New Faculty Startup Research Fund (Grant No. 5110105) from Tianjin University.

JF0632740