Is Medical Control of Herbal Tea Consumption Necessary?

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Abstract

   Chinese Beiqishen tea extract was studied in an in vitro test system. Phytochemical screening and trace element analysis as well as the detection of antioxidant properties have been carried out. Total polyphenol content of the tea extract (2 g/250 ml) was 83.2 mg/100 ml, kaemferol content: 1.99 mg/100 ml and quercetin content: 0.75 mg/100 ml. Caffeine was the predominant compound responsible for refreshing. Element analysis showed relative high concentration (mg/100ml) of Al (0.562), B (0.0136), Ca (1.714), Cr (0.0006), Cu (0.0061), Fe (0.0136), K (15.92), Mg (1.465), Mn (0.44), Ni (0.0064), P (1.454) in the tea extract. Proton-donating ability (I_{50}=0.1ml), reducing power (7.78 ascorbic acid equivalent·ml^{-1}) and total scavenger capacity ($\approx$99 % in 0.1 ml volume) were identified as a function of concentration. Total antioxidant status (TAS) was 8.7 mmol/L trolox equivalent. Based on the results the tea infusion contains trace elements and caffeine in addition to polyphenols and tannins in high concentration, therefore the attention of patients suffering from gastrointestinal, renal and cardiovascular diseases, as well as in pregnancy should be called to the fact that consumption of the tea without control involves health risks.

INTRODUCTION

   Ancient natural therapeutical methods have been discovered by modern food and drug investigations. Tea consumption has an extremely high effect on primary and secondary prevention and on the improving the quality of life, because higher plants have many free radical scavenger molecules and anti-inflammatory compounds. In general, the antioxidant, immunstimulant and anticarcinogenic properties of those natural substances are widely promoted. In most cases, tea infusion contains a number of active molecules without scavenger properties as well as trace elements in high concentration.

   The main problem is that traditional popular medicinal procedures e.g. tea consumption, generally leaves genetic polymorphism of human out of consideration. Another concern is that patients do not take into consideration the fact that these natural products contain concentrated active components as well. Therefore serious complications may arise related to herbal medication (Jadoul et al., 1993; Vanherweghem et al., 1993; Buth 1993; Gartner et al., 1993).

   According to the information sheet of commercially available Beiqishen tea imported from Far East, it may be assumed that its active compounds have antioxidant and/or free radical scavenger properties and these molecules can influence or modify the regulation of the cell cycle. In contrary to the information given on the tea bags, the Internet examinations (http.//www.beiqishen.com/) identified root and stalk of *Astragalus membraneceus, Ganoderma lucidum* (mushroom) and the fruit of *Lycium species*

(Chinese wolfberry) and *Camellia sinensis* as ingredients (Héthelyi et al., 2001).

In this study we give account of our further analytical examinations and study the probable effects of active compounds on tissue redox homeostasis.

MATERIALS AND METHODS

Beiqishen tea (Daxing' anling Beiqishen Healthy Food Co, Ltd., Permission number No. 022; 1998) obtained from the commercial network was examined to determine its antioxidant property and to control its main active components. By the prescription, this product was made from the roots, tender stalks, leaves and flowers of wild *Astragalus mongolicus* growing in Danxing' anling, supplemented with Glossy ganoderma and the fruit of Chinese wolfberry. Recommended dosage: one bag each time three times a day. Immerse bag in boiling water for five minutes. These active compounds ensure beneficial effect on several diseases.

Tea making: Ten-ten packets of tea taken from different boxes of Beiqishen tea drug were mixed and 2 g of which were infused with 250 mL of boiling double distilled water and allowed to stand for 30 min.

The polyphenol content of the drug and extract was measured according to the Hungarian Pharmacopoeia (Edition VII, 1992.) by spectrophotometric method at 750 nm, using pyrogallol as reference standard. Flavonoid content in the sample was determined spectrophotometrically according to the German Pharmacopoeia (Deutsches Arzneibuch, DAB10, 1996). Glycosides and aglycones were determined together in aglycone form. Tannin was determined quantitatively by its absorption on standard hide powder (Hungarian Pharmacopoeia, Edition VII. 1992.).

The flavonols (quercetin, kaemferol) in the tea extract were measured as aglycons according to Hertog and coworkers (1992). Flavonoid glycosides were extracted and hydrolyzed to their aglycons with 6.0N HCl in boiling 62.5 % aqueous methanol in the presence of 0.1 g t-butylhydroquinone for two hours. After refluxing the extract was allowed to cool and was subsequently made up to 50 mL with methanol and sonicated for 5 min. Approximately 2 mL was filtered through 0.45μm filter (Chromafil AO-20/25) before injection. The resulting aglycons were quantified by RP-HPLC (Perkin Elmer) on a Premisphere C_{18} column (150 x 3.9 mm, 5 μm, Phenomenex, USA (365 nm). Calibration curves of individual flavonoids were made over a range of 5-20 μgmL^{-1}. Detector response was linear over the concentration range used. For all standards r^2 was higher than 0.998 (Lugasi and Hóvári, 2000).

Preparation of the tea for gas-chromatographic methods was done by the cold extraction method with $CHCl_3$, and/or EtOH solution. Of this tea, 6-8 g was filtered in 3 x 30 ml solvent and rotavapored at 40 ^{o}C (Héthelyi et al., 2001).

Gas-chromatographic (GC) measurements were done with Shimadzu GC-14 B gas-chromatography equipment. Fused silica capillary column: 30 m x 0.25 mm ID x 0.25 μm SE-30 film thickness was used. IB: 220 ^{o}C; FID detector temperature 250 ^{o}C, oven temperature: 110 ^{o}C (3 min), 8 ^{o}C/min to 220 ^{o}C (5 min) analysis time: 21.45min. Nitrogen carrier gas 99.999 % about 1mL/min; slitter 75:1 splitter; Shimadzu Class–VP Chromatography Data System were applied. (Héthelyi et al. 2001).

Caffeine was identified by the GC/MS method done on a Finnigan-Mat GCQ Ion Trap instrument: 30 m x 0.25 mm ID x 0.25 μm, Restec 5 capillary column was applied, the mass spectra were recorded in the range m/z 30-650, analysis time was 42 min. The system was controlled by IBM PC (Gateway 2000), with the use of helium 6.0 as carrier gas, 67:1 split. The components determined were identified by matching the EI -spectra against the NIST library (Finnigan Corp. San Jose, CA).

Element concentrations in the tea extracts were determined by inductively coupled plasma optical emission spectrometry (ICP-OES). The type of the instrument was a sequential plasma emission spectrometer (Atom Scan 25, Thermo Jarrell Ash). The following 23 elements were determined: Al, As, B, Ba, Ca, Cd, Co, Cr, Cu, Fe, Hg, K, Li, Mg, Mn, Mo, Na, Ni, P, S, Ti, V, Zn. Three times three sec. integration time, blank subtraction and background correction were applied during the measurements. Sample

preparation for element measurement was following: tea samples (2.0 g) were digested with a mixture of HNO_3 (5 mL) and H_2O_2 (3 mL) in teflon vessels. After digestion the samples were diluted to 25 mL with deionized water.

Proton-donor activity was determined by Blois's method (1958), modified by Blázovics et al (1999) in the presence of a 1,1-diphenyl-2-picryl-hydrazyl radical. Absorbance of the methanolic DPPH-dye was assessed spectrophotometrically at 517 nm. For characterization of the activity, inhibition percentage was given to the DPPH degradation.

Oyaizu's method was adopted for analysis of the reducing power. The change in absorbance was measured, which accompanied Fe^{3+}- Fe^{2+} transformation at 700 nm, and the reducing power property was compared to that of ascorbic acid (Oyaizu, 1986).

The chelating activity of tea extract on copper (II)–ions was measured according to Shimada et al. (1992). Aliquots of sample were added to hexamine buffer (1.0 mL, 10 mM, pH=5.0) containing KCl (10 mM) and $CuSO_4$·5 H_2O (3 mM). The reaction mixture was shaken and after 2 min tetramethylmurexide (0.1 mL, 1 mM) was added. Absorbances at 485 and 530nm were recorded. Chelating activity was expressed as the absorbance ratio at 485 vs 530 nm. Absorbance ratio of the reaction mixture containing 1ml of distilled water was 3.55 ± 0.05. Samples having lower absorbance ratios than 3.55 exhibit chelating activity.

The total scavenger capacity was determined by chemiluminescence method in a H_2O_2/·OH-luminol-microperoxidase system. Total volume of reaction mixture system was 1ml. The chemiluminescence light intensity given in RLU (relative light unit) is reduced in the presence of free radical scavenger compounds (Blázovics et al., 1999).

The determination of total antioxidant status (TAS) was carried out using the Randox diagnostic kit with a COBAS MIRA automatic laboratory analyzer. The measurement is based on the procedure written by Miller and coworkers (1993).

1,1-diphenyl-2-picryl-hydrazyl stable radical, tetramethylmurexide, luminol, microperoxidase and hydrogen peroxide were obtained from SIGMA (St.Louis), Merck ICP standard was obtained Merck (Darmstadt) all other reagents were purchased from Reanal (Budapest).

Mean values and standard deviation (SD) were calculated from the results. Microsoft Excel 97 program was used for examination. Each meaning point represents five parallel data in redox experiments. The figures show mean values where c.v.% was below 5.00 %. Significance level was determined at $P < 0.05$.

RESULTS AND DISCUSSION

Over the past few years several papers have been published on the important role of oxidative stress on living cells and cell responses e.g. apoptosis and necrosis, which leads to cell death (Powis et al., 1997; Morel and Barouki, 1999; McCord, 2000; Rust and Gores, 2000).

Oxygen free radicals may damage a wide variety of organic compounds, e.g. DNA, proteins, lipids, carbohydrate, different small molecules in living organisms and cause cell death, apoptosis or necrosis. At the same time mild oxidative stress can modulate signal transduction cascades and redirect gene expression, and influence many cellular responses, e.g. proliferation, differentiation, reproduction. Regulations of the cell cycle depend on intracellular redox state. Reduced glutathione and thioredoxin are key factors in intracellular redox homeostasis. Critical steps in the signal transduction cascade are sensitive to oxidative stress and antioxidants. Levels of Bcl2 protooncogene and p53 protein influence the apoptotic death of cells and tumorigenesis. Apoptosis may be considered as programmed cell death characterized by organized nuclear and cellular fragmentation, but necrosis involves dramatic tissue destruction (Powis et al., 1997; Rust and Gores, 2000).

Many research laboratories give account of the anticarcinogenic effect of polyphenols and flavonoids in different tumor cell cultures, as well as their anti-inflammatory and antioxidant activities in experimental studies (Sakagami et al., 2000;

Blázovics et al. 2001; Hagymási et al., 2001). Although a lot of polyphenols, especially tannins precipitate proteins, depending on the dose, and therefore cannot penetrate the mucous membranes (Lugasi, 2000).

Polyphenols (20.77 ± 0.52 g /100 g drug), tannins (9.063 ± 0.783 g/100 g drug) and flavonoids (0.485 ± 0.036 g/100 g drug), the main antioxidant constituents of Beiqishen tea drug resulted in a significant antioxidant capacity (Blázovics et al., 2002). Total polyphenol content of the tea extract was 83.2 mg/100 mL. Significant amount of quercetin and kaempferol could be detected (0.75 and 1.99 mg/100 mL respectively). Beiqishen tea extract exerted proton-donating ability in the presence of DPPH stable radical as a function of concentration. $I_{50}=0.10$ ml tea extract which is the amount needed for the 50 % inhibition of the color development of DPPH. For comparison ascorbic acid $I_{50}=0.013$mg and trolox $I_{50}=0.018$mg are in this system (Blázovics et al., 2002). The tea sample exhibited reducing power, which was measured on the basis of Fe(III) to Fe(II) redox reaction. The reducing power was expressed in ascorbic acid equivalent (ASE/ml). ASE/ml =1ml sample exhibited the same reducing power, that 7.78 µmol ascorbic acid (Blázovics et al., 2002).

The chelating activity of Beiqishen tea extract was justified as well (Table 1.).

Strong scavenger capacity (≈99 % in 0.1ml volume) was also verified against oxygen free radicals in a medium consisting of hydrogen peroxide and hydroxyl radicals. The scavenging activity of the sample was determined in a H_2O_2/˙OH-microperoxidase-luminol system (standard solution) by a chemiluminometric method. The emitted light of this system diminished with the antioxidant components of tea infusion as a function of concentration, as can be seen in Figure 1. Higher chemiluminescent intensity (in RLU) than the intensity of standard solution (10654732 ± 15408 RLU) points to metallic ion catalysis. Total antioxidant status was 8.7 mmol/L trolox equivalent, which corresponded with chemiluminescent results.

Metallic element analysis showed that Al, B, Cr, Co, Fe, Mn and Ni content in the aqueous extract was higher than that in other tea samples (Szentmihályi et al., 1999; Szentmihályi et al., 2000; Máday et al., 2000). The dissolving rate (20-98%) indicated that the elements could be found in water-solving form (Table 2). Metal ions enhance the production of TNF-alpha and activate protein kinase C and influence the reduction of stress proteins. The presence of Zn, Cu, Cd, Mn and Ni divalent cations can modulate the function of tumor suppressor protein p53 in vitro (Kernohan et al., 1996). Competition between Ni(II) and Mg(II) may provide an important mechanism for interfering with DNA-protein interactions involved in the repair process, because the inhibition of DNA repair is partly reversible by the addition of Mg(II) (Kasprzak et al., 1987., Hartwig et al., 1994). In addition, presumable Ni(II), Co(II) and As(II) ions displace Zn ion in the zinc-finger structure of DNA repair enzymes (Hartwig, 1998). Ni, Cr and As elements are established carcinogens, and Si and Ti are suspected carcinogens in humans (Hayat, 1996; Hadfield et al., 1998). Ni and Fe can jointly induce renal sarcomas (Higinbothan et al., 1992). Mg and Zn were found to diminish the acute toxic effect of Ni. As is known, Mg inhibits Ni uptake by the target tissues, and diminishes Ni induced muscle tumor formation. Ca ions in most cases do not prevent carcinogenesis and do not influence the uptake of toxic metal elements (Kasprzak et al., 1987). Fe, Cu and Cr undergo redox cycling, and Ni depletes glutathione and protein-bond SH-groups resulting in the production of reductive oxygen species (Stohs and Bagchi, 1995). Lipid peroxidation processes can be induced in membranes under toxic metal ion power (Blázovics et al., 2001). At the same time in oxidative stress metallothionein genes can be activated and the level of these Zn dependent and cisteine-rich metal-binding proteins are increased. Toxic metal elements also induce the gene expression of metallothioneins and than these proteins bond excess metal ions (Veness-Meehan et al., 1991). An experimental study verified that Zn-metallothioneins influenced the subcellular distribution of trace elements in hepatocytes (Liu et al., 1991). Metallothionein may also sequester Fe, thus preventing its participation in free radical reactions (Powis et al., 1997).

On the basis of our examinations, it may be established that the predominant

compound for refreshing effect is well absorbing caffeine. In the examination of the Beiqishen tea extract by the GC-method, the GC-chromatogram showed a high amount (30-36 %) of the compound with $\lambda=272$ nm absorbance maximum. Mass-spectrum revealed that this peak is caffeine (mw 194, retention time 21 min) identified by NIST library. Caffeine is a very effective inhibitor of apoptosis. Caffeine and metallic elements, especially As, Cr, Ni and Fe have effects opposite to those of phenolic antioxidants.

CONCLUSION

Possible synergistic and antagonistic effects of the compounds may be assumed. Natural preparations may themselves be toxic and especially dangerous with interaction of other medicines. Herbal tea consumption must be controlled by experts especially in the case patients with high risk of tumorigenesis, nephropathy, cardiovascular or gastrointestinal diseases. Frequent drinking of herbal teas is contraindicated in pregnancy, lactation, and childhood.

ACKNOWLEDGEMENTS

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Tables

Table 1. Copper (II) – chelating activity of Beiqishen tea extract.

Samples (ml)	Absorbance ratio (485 vs 530 nm)
Control	3.50 ± 0.03
0.2	2.63 ± 0.01
0.5	2.21 ± 0.02
1.0	1.86 ± 0.01

Table 2. Element concentration in Beiqishen tea extract and dissolution rate from Beiqishen tea drug into the tea extract.

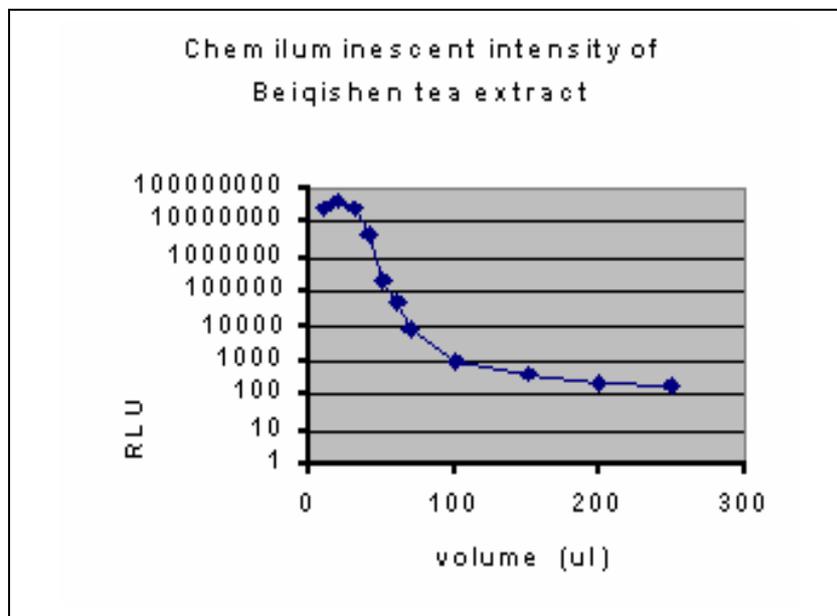| Elements	mg/100 ml	Dissolution rate (%)
Al	0.562 ± 0.023	30.38
As	<0.00025	-
B	0.0136 ± 0.001	97.28
Ba	0.0061 ± 0.0004	19.80
Ca	1.714 ± 0.048	24.55
Co	0.00035 ± 0.00014	42.06
Cr	0.0006 ± 0.0003	16.41
Cu	0.0061 ± 0.0009	26.36
Fe	0.0136 ± 0.001	3.19
K	15.92 ± 0.57	76.59
Mg	1.465 ± 0.005	60.21
Mn	0.44 ± 0.008	36.08
Na	0.265 ± 0.02	85.19
Ni	0.0064 ± 0.0004	86.85
P	1.454 ± 0.056	54.48
S	1.68 ± 0.035	46.87
Ti	0.0002 ± 0.0001	1.59
Zn	0.0144 ± 0.0003	36.67

Figures

Fig. 1. Total scavenger capacity of Beiqishen tea extract.