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The effect of black tea on risk factors of cardiovascular disease in a normal population

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ABSTRACT

Objectives. A prospective randomized controlled clinical trial determined the effect of Mauritian black tea consumption on fasting blood plasma levels of glucose, lipid profiles and antioxidant status in a normal population.

Methods. The study group (71%) consumed 3 x 200 ml of black tea infusate/day for 12 weeks without additives followed by a 3 week wash-out. The control group (29%) consumed equivalent volume of hot water for same intervention period.

Results. The tea used had high levels of gallic acid derivatives (50 ± 0.4 mg/L), flavan-3-ols (42 ± 2 mg/L), flavonols (32 ± 1 mg/L) and theaflavins (90 ± 1 mg/L). Daily 9 g supplementation of black tea infusate induced, in a normal population, a highly significant decrease of fasting serum glucose (18.4%; $p<0.001$) and triglyceride levels (35.8%; $p<0.01$), a significant decrease in LDL/HDL plasma cholesterol ratio (16.6%; $p<0.05$) and a non significant increase in HDL plasma cholesterol levels (20.3%), while a highly significant rise in plasma antioxidant propensity (FRAP: 418%; $p<0.001$) was noted .

Conclusion. Black tea consumed within a normal diet contributes to a decrease of independent cardiovascular risk factors and improves the overall antioxidant status in humans.

Introduction

Tea (*Camellia sinensis*) is popular drink worldwide linked to good health. Black tea contains relatively high levels of polyphenolics with the major phenolics being the flavan-3-ols ((epi)catechins, (epi)gallocatechins and their gallate esters), the flavonols (mono-, di-, and tri-glycoside conjugates of myricetin, quercetin and kaempferol) the flavones and quinic acid esters of gallic, coumaric and caffeic acids. Black tea has a reduced flavan-3-ol monomer content and higher levels of their polymerized derivatives theaflavins, which account for about 10-30% of the converted catechins, and thearubigins (de Mejia et al., 2009; Rouanet et al., 2010). Although there is a growing interest in the hypothesis that tea has a preventive effect against cardiovascular diseases and that tea polyphenols may mediate the observed benefits (Sharangi, 2009; Stangl et al., 2006), the intricate mechanisms of polyphenolic action still need to be comprehensively understood.

The potentiality that tea consumption can reduce the risk of cardiovascular diseases and total mortality can be deduced from epidemiological studies (Sharangi, 2009; Stangl et al., 2006), but data are not uniform and consistent. This may be due to confounding factors including varying socioeconomic status of participants, dietary habit and lifestyle, and difference in experimental protocols (Pietta et al., 1998; Serafini et al., 1996; van het Hof et al., 1997). Also many of these studies have mainly targeted populations with established pathologies, with few investigations reported in normal populations. The objective of this study was to determine the effects of black tea consumption on fasting serum glucose, total cholesterol, triglycerides, HDL, LDL and antioxidant status in a normal population.

Methods

Subjects

Sample size was limited by usual constraints (i.e. cost, time availability and availability of volunteers to satisfy the selection criteria). A sample size determination software (http://www.statisticalsolutions.net/pss_calc.php) was used to ensure a minimum power of 0.8. 87 volunteers, not following any form of therapy, were recruited by the research team, based on the

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following criteria: gender, age group from 25–60 yrs old, non-smoker or former smokers who had stopped for less than 6 months, alcohol intake (less than 2 standard drinks/day), postmenopausal women not receiving hormone replacement therapy and ejection fraction greater than 40%. Written informed consent was obtained from all volunteers prior to the study, which was conducted following approval by the Ministry of Health and Quality of life National Ethics Committee (Republic of Mauritius).

Study Design

The study consisted of a randomized, controlled clinical trial (clinicaltrial.gov Identifier NCT00114907) with treatment and control groups set in parallel in the ratio of 7:3, over a 15-week intervention period. The random allocation sequence was generated by a qualified statistician using a random generator that keeps in view ratio allocation to each group as well as age and gender distribution. A simple randomization approach was adopted with no blocking, with particular care to maintain the power of the design for each group. The treatment group consumed 3 x 200 ml of black tea infusate/day (3 standard cups of 200 ml hot water each containing 3 g of black tea (infused for 5 minutes)) for 12 weeks without additives (milk or sugar) followed by a 3 week wash-out period by consuming same volume of hot water/day. Tea bags were purchased from Mauritius Corson Tea Estate Co Ltd. The control group consumed equivalent volume of hot water for same intervention period. Subjects were maintained on their normal diet and were required to fill a diet survey form over the study period.

Blood collection and preparation for sample analysis

Blood collection was made at the Cardiac Centre of the SSRNH, Pamplemousses. Subjects were requested to fast for at least 10 hours before blood collection. 15 ml of fasting blood were collected and dispensed into 4 different tubes comprising 2 heparinized tubes (2x4 ml), fluoride oxalate tube (2–5 ml) and plain tubes. Fresh blood samples were preserved in icebags for analysis. Fluoride oxalate tubes were centrifuged at 3000 rpm for 10 mins and supernatant removed for determination of fasting glucose level using reagent kits. Heparinized tubes were centrifuged and plasma removed for determination of total cholesterol, triglycerides, HDL, LDL and antioxidant status using the TEAC and FRAP assays.

Analysis of biomarkers and Plasma total antioxidant activity

All analyses were made in either clear blood serum or plasma samples after centrifugation at the University of Mauritius The automated HumaStar 80 apparatus was used for colorimetric analysis of all biomarkers maintained at 37 ° C. Reagent kits for total cholesterols, low density lipoproteins (LDL) and high density lipoprotein (HDL) measurements were from Human Co (Weinsbaden, Germany), for glucose and triglycerides from Biosystem (Barcelona, Spain). Reagent kits for measurement of Trolox equivalent antioxidant capacity (TEAC) in blood plasma were from RANDOX (Crumlin, UK). The Ferric–reducing ability of plasma (FRAP), which measures the ability to donate electrons, was assessed with the method of (Benzie and Strain, 1996). Units for both TEAC and FRAP are in millimole activity per litre of plasma

Survey of diet forms

Compliance to protocol was assessed by a follow-up of the daily diet of the volunteers. A dietary questionnaire indicating food items consumed daily during the three main meals was issued to each subject and was collected dully filled after blood sampling exercise from each volunteer. This Information enabled the assessment of any possible changes in the diet during the study. Statistical analysis of these forms consisted of a ranking strategy based on the fat/lipid value (USDA Food Composition database, 2006) of each food item consumed by both study group and control group (e.g. a food item high in fat content (e.g. red meat) would score 10/10 marks while a vegetable low in fat would be assigned 1/10; the mean value in arbitrary units obtained per day being further averaged on a weekly basis and the trend observed during the 15-week intervention period)

Determination of total phenolic, flavonoid and proanthocyanidin contents of black tea extract

The total phenolic content of black tea infusate was estimated using the Folin-Ciocalteu method adapted from Singleton and Rossi (1965). Results are expressed in mg of gallic acid equivalent g^{-1} fresh weight of plant material. The $AlCl_3$ method adapted from Lamaison and Carnet (1990) was used for the determination of the total flavonoid content of the tea infusate. Flavonoid contents are expressed in mg quercetin equivalent g^{-1} fresh weight of plant material. The modified acid/butanol assay of (Porter et al., 1986) was used to quantify the total proanthocyanidin content of the tea infusate. Results are expressed in mg of cyanidin chloride g^{-1} fresh weight.

HPLC-PDA-MS^2 analysis of phenolic derivatives from black tea

The black tea was analyzed for its phenolic derivatives by the HPLC-PDA-MS^2 system essentially as described in Del Rio et al. (2004). The tea sample was prepared in accordance with the protocol given to the volunteers i.e. by infusion of one tea bag (3 g) in 200 ml of boiling water for 5 minutes. Aliquots of the tea extract were taken, allowed to cool down and spun on a bench micro-centrifuge at 6000 *g*. The supernatant was directly injected into the HPLC-PDA-MS^2 system.

Statistical Analysis

Simple regression analysis was performed to calculate the dose–response relationship of standard solutions used for calibration as well as test samples. The Unicam Vision 32 software (Unicam Ltd, UK) was used to evaluate initial and final antioxidant rate values for FRAP assay. Plasma antioxidant data (TEAC and FRAP) were recorded on Microsoft Excel. After data cleaning, statistical analyses were carried out using both Microsoft Excel and SPSS 13.0 statistical software. Statistical analysis of data involved descriptive a well as inferential statistics. For descriptive statistics tables of summary statistics including mean, standard deviation, percentage, minimum and maximum values as well as graphs such as histograms and bar charts with error bars and box plots were used. Prior to inferential analyses, choices of tests were based on normality of data. Where data were normal (Shapiro-Wilk's test not significant, i.e. $p > 0.05$) parametric tests were used. Tests of significance of observed mean differences over the intervention period for 2 sets of data were performed using Student's *t*-test and where data were not normal the non-parametric alternative Mann–Whitney *U* test was used. Kruskal Wallis test was used as a non-parametric alternative for one-way ANOVA for comparing more than 2 sets of data. The critical limits for test of significance were set at 5%, 1% and 0.1% successively.

Results

Phenolic analysis of tea infusate

Total phenolics, flavonoids and proanthocyanidins of the tea infusates were respectively 89 ± 25 mg gallic acid/g dry weight, 21 ± 7 mg quercetin/g dry weight and 35 ± 5 mg cyanidin chloride/g dry weight. HPLC-MS^2 analysis of tea clearly suggests a a complex phenolic profile (Fig. 1). The feature identified include, 5-galloylquinic acid (Peak 2), (+)-gallocatechin (Peak 3), catechins and derivatives ((+)-gallocatechin (peak 3), (−)-epigallocatechin (peak 4), (+)-catechin (peak 5), (−)-epicatechin (peak 7) (−)-epigallocatechin gallate (peak 8), (−)-epicatechin gallate (peak 13), (six quercetin glycosides (Peaks 9–12, 14 and 15), three unknown quercetin derivatives (Peaks 6, 22 and 25), four kaempferol glycosides (Peaks 17–20), three unknown kaempferol derivatives (Peaks 16, 23 and 28) and theaflavin and its derivatives (Peaks 21, 24, 26 and 27),

Quantification using appropriate standard curves showed that total gallic acid derivatives in the tea infusate amounted to 50 ± 1 mg/L. Total flavan-3-ols and flavonols were 42 ± 2 mg/L and 32 ± 1 mg/L respectively while the total theaflavin content was 89 ± 1 mg/L . The TEAC and FRAP values were of the order of 1055 ± 25 and 825 ± 23 μmol/g dry weight respectively.

Study subjects

The number of subjects at the end of the 15-week intervention period was 77 representing a 12% drop-out from the initial population size. Reasons for drop-out were occupational and familial constraints. There was no incidence of pathological events during that period. The