



HAL
open science

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

Theeshan Bahorun, Amitabye Luximon-Ramma, Vidushi Neergheen-Bhujun, Teeluck Kumar Gunness, Kreshna Googoolye, Cyril Auger, Alan Crozier, Okezie I Aruoma

► To cite this version:

Theeshan Bahorun, Amitabye Luximon-Ramma, Vidushi Neergheen-Bhujun, Teeluck Kumar Gunness, Kreshna Googoolye, et al.. The effect of black tea on risk factors of cardiovascular disease in a normal population. Preventive Medicine, 2012, 54 (Supplement 1), pp.S98-S102. 10.1016/j.ypmed.2011.12.009 . hal-00705025

HAL Id: hal-00705025

<https://hal.science/hal-00705025>

Submitted on 15 Nov 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

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

Theeshan Bahorun ^{a,*}, Amitabhe Luximon-Ramma ^a, Vidushi S. Neergheen-Bhujun ^b,
Teeluck Kumar Gunness ^c, Kreshna Gogoolye ^a, Cyril Auger ^d, Alan Crozier ^e, Okezie I. Aruoma ^{e,**}

^a ANDI Centre of Excellence for Biomedical and Biomaterials Research, CBBR Building, MSIRI, University of Mauritius, Réduit, Republic of Mauritius

^b ANDI Centre of Excellence for Biomedical and Biomaterials Research, CBBR Building, MSIRI and Department of Health Sciences, Faculty of Science, University of Mauritius, Réduit, Republic of Mauritius
^c Cardiac Centre, Sir Seewoosagur Ramgoolam National Hospital (SSRNH), Pamplemousses, Republic of Mauritius

^d Plant Products and Human Nutrition Group, Division of Environmental and Evolutionary Biology, Institute of Biomedical and Life Sciences, University of Glasgow, Glasgow, Great Britain

^e School of Biomedical Sciences and School of Pharmacy, American University of Health Sciences, Signal Hill, USA

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

* Correspondence to: T. Bahorun, National Research Chair, ANDI Centre of Excellence for Biomedical and Biomaterials Research, CBBR Building, MSIRI, University of Mauritius, Réduit, Republic of Mauritius.

** Correspondence to: O.I. Aruoma, School of Biomedical Sciences and School of Pharmacy, American University of Health Sciences, Signal Hill, USA.

E-mail addresses: tbahorun@uom.ac.mu (T. Bahorun), oaruoma@auhs.edu (O.I. Aruoma).

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 (clinical-trial.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 cholesterol, 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 AlCl₃ 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-MS² analysis of phenolic derivatives from black tea

The black tea was analyzed for its phenolic derivatives by the HPLC-PDA-MS² 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-MS² 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 as 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-MS² analysis of tea clearly suggests 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

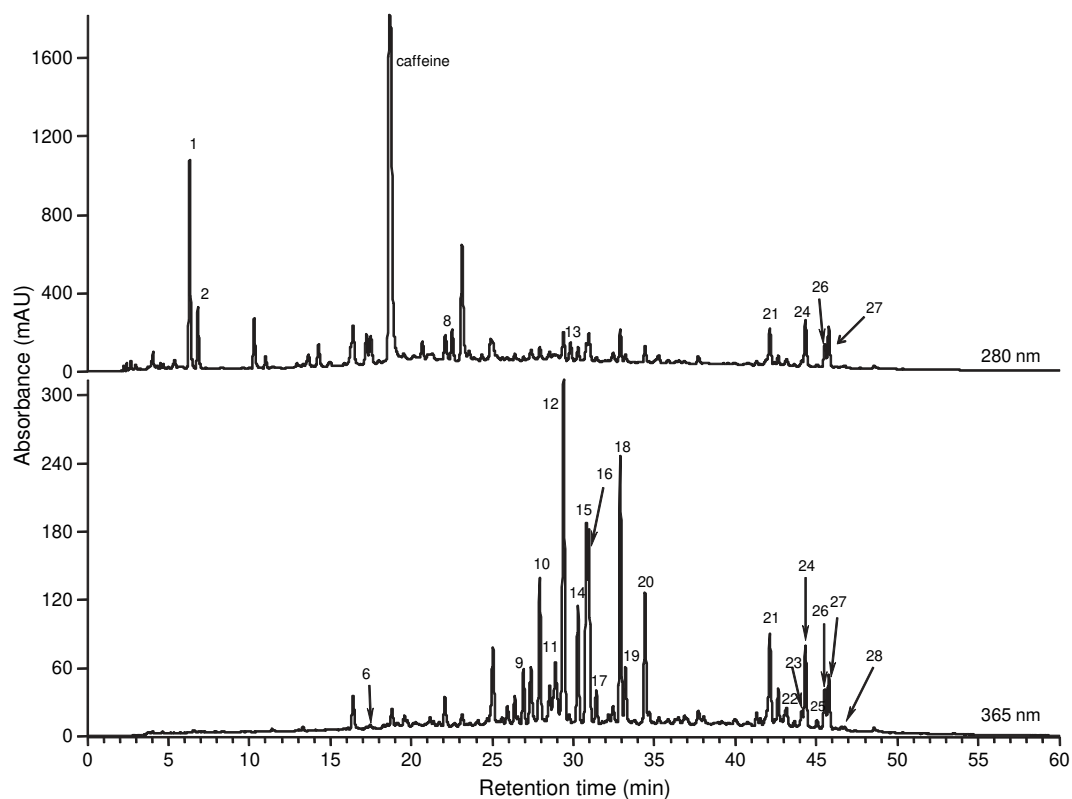


Fig. 1. HPLC-MS² profile of the Mauritian Golden Pekoe black tea at 280 nm (black trace A) and 360 nm (red trace B). 1: gallic acid; 2: 5-galloylquinic acid; 3: gallic acid; 4: epigallocatechin; 5: catechin; 6: unknown quercetin derivative; 7: epicatechin; 8: epigallocatechin gallate; 9: quercetin-rhamnosylgalactosylglucoside; 10: quercetin-3-rutinosylglucoside; 11: quercetin-rhamnosylglucoside; 12: quercetin-3-rutinoside; 13: epicatechin gallate; 14: quercetin-3-galactoside; 15: quercetin-3-glucoside; 16: unknown kaempferol derivative; 17: kaempferol-rutinoside; 18: kaempferol-3-rutinoside; 19: kaempferol-galactoside; 20: kaempferol-3-glucoside; 21: theaflavin; 22: unknown quercetin derivative; 23: unknown kaempferol derivative; 24: theaflavin-3-gallate; 25: unknown quercetin derivative; 26: theaflavin-3'-gallate; 27: theaflavin-3-3'-digallate and 28: unknown kaempferol derivative.

final sample population consisted of 42 (55%) males and 35 (45%) females in the age group of 25–60 years old. The levels of biomarkers measured under both tea and control regimens at the beginning of the study, its percentage change at the end of the intervention period and after a three week wash-out period were recorded.

The Diet scores

The compiled diet scores calculated as per the lipid/fat contents of the food items consumed during the study varied from 28 to 218 arbitrary units at baseline analysis. The mean data show that the scores remained relatively constant for both the tea and control groups in the combined male and female population from baseline to wash-out period (Fig. 2).

Fasting blood glucose concentration

The mean fasting blood glucose level, recorded at baseline, was 134 ± 66 mg/dL for males and 111 ± 38 for female subjects. These levels were greatly influenced by tea consumption with a significant drop of 20.3% and 14.8% ($p < 0.001$) respectively after 12 weeks (Table 1). Wash-out treatment generally increased the blood glucose level in the study groups. Non significant decreases (12.6% and 22%) were also observed in the control groups with subsequent non significant increases with the wash-out treatments.

Lipid profiles

Tea consumption did not significantly influence total cholesterol (TC) levels in male subjects while an increase of 14% ($p < 0.05$) was

measured in the female population (Table 1). The wash out of the study group resulted in very slight non significant increases compared to the 12th week levels. Very slight non-significant increases were also observed in the control groups.

The triglycerides (TG) baseline data for men and women in the tea group were 166 ± 138 mg/dL and 102 ± 65 mg/dL. These values were significantly reduced by 38.6% and 28.6% ($p < 0.01$) following tea intake (Table 1) Wash out significantly increased TG levels recorded at week 12 by 25% and 24% ($p < 0.05$) respectively. In the control group non significant decreases of TG levels were noted for both males and females after 12 weeks followed by non significant increases after the wash out period.

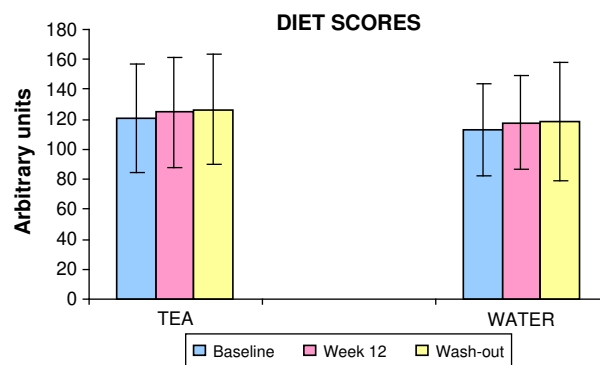


Fig. 2. Variation in the diet scores for volunteers under the tea and water regimens during the 12 week intervention period and 3 weeks wash-out treatment in a combined male and female Mauritian population. Data represent mean values (bars) with standard errors for study group (left: n = 155) and control group (right: n = 55).

Table 1

Mean (SD) values of blood biomarkers measured during the 12-week intervention period and 3-week wash-out period in a male and female Mauritian population (N = number of subjects) under the tea and control regimens. Significance testing between baseline and week 12 values and wash-out period for both study group and control group was performed by Student's *t*-test comparing mean values of two samples. **p*<0.05 (significance at 5%), ***p*<0.01 (significance at 1%), ****p*<0.001 (significance at 0.1%).

Tea Regime					Control			
Gender	N	Mean (SD) Baseline value	Mean (SD) Week 12 value	Mean (SD) Wash-out value	N	Mean (SD) Baseline value	Mean (SD) Week 12 value	Mean(SD) Wash-out value
Blood glucose level (mg/dL)								
Male (Normal)	33	134.9(67)	107.5(48.1) ***	115.6(51.9)	9	116.3(59.6)	101.7(50.6)	103.7(57.7)
Female (Normal)	22	111.4(38.8)	95(31.4) ***	107.1(46.7)	13	131.7(54.1)	102.8(39.4)*	125.4(75)
Triglycerides level (mg/dL)								
Male (Normal)	33	166.6(138.3)	102.2(60.8)**	127.8(69.3)*	8	130.6(83.8)	93.9(29.4)	98.4(38.3)
Female (Normal)	21	102.4(65.5)	73.1(54.7)*	90.6(56.9)*	12	130.4(84.6)	52.5(29.9)	85.8(24.0)*
Total cholesterol level (mg/dL)								
Male (Normal)	30	161.8(30.6)	167.2(40)	175.7(30.5)	8	161.6(22.6)	167.1(27.5)	170.8(28.8)
Female (Normal)	22	157.9(33.6)	180(39.6)*	180.5(45.3)	12	171.8(24.6)	176.4(35.8)	177.5(30.1)
LDL cholesterol level (mg/dL)								
Male (Normal)	31	107.7(46.1)	105.5(33.7)	116.8(31.9)	8	111.9(48.3)	124(22.8)	123.3(28.5)
Female (Normal)	22	96.0(46.5)	110.1(33.5)	118.0(37.8)	11	97.3(29.7)	108.6(27.6)	123.3(31.9)
HDL cholesterol level (mg/dL)								
Male (Normal)	29	42.6(15.0)	49.9(9.8) *	57.4(12.4) ***	8	57.9(23.3)	55.1(8.0)	62.3(13.3)
Female (Normal)	22	43.6(15.2)	54.2(8.4) *	69.9(10.6) ***	12	35.9(13.2)	53.8(9.4) ***	62.8(12.0) *
Mean values of LDL:HDL cholesterol ratio								
Male (Normal)	29	2.6(1.3)	2.2 (0.8)*	2.1(0.6)	8	2.1(1.4)	2.3(0.5)	2.0(0.5)
Female (Normal)	22	2.5(1.5)	2.1(0.7)*	1.7(0.6) **	12	3.0(1.3)	2.0(0.6) *	2.0(0.5)
FRAP activity (mmol/L)								
Male (Normal)	31	1.1(0.6)	6.1(1.3) *	9.7(2.0) **	7	1.1(0.3)	6.7(1.4) **	10.6 (2.2)
Female (Normal)	20	1.2 (0.8)	5.7(1.1) *	9.1(1.7)	8	1.2 (0.4)	6.3(1.2)	10.0 (2.0)**
TEAC activity (mmol/L)								
Male (Normal)	29	2.3(0.2)	2.2(0.2) *	2.0(0.2) **	8	2.2(0.2)	1.9 (0.2) **	2.1(0.3)
Female (Normal)	19	2.4(0.5)	2.2(0.2) *	2.2(0.3) **	9	2.1(0.5)	1.8(0.1)	2.6(0.3)

A slight non significant reduction of low density lipoprotein (LDL) cholesterol (2%) was noted in the male study group while a non-significant increase (14.6%) occurred among the female subjects (Table 1). Wash out increased the LDL levels non significantly in both male and female. In the control group hot water consumption induced non-significant increases of 10.8% and 11.6% during the intervention period.

Baseline HDL levels for males and females in the study group were 42 ± 15 mg/dL and 43 ± 15 mmol respectively. Tea consumption increased these levels by 17% (*p*<0.05) and 24% (*p*<0.05) (Table 1). High significant increases of 15% and 29% (*p*<0.001) were recorded after the wash-out. No significant changes were recorded in the male control group while in the female control group hot water consumption induced a highly significant increase (50% : *p*<0.001) after 12 weeks and this rise in HDL levels continued during the wash out period (+ 16.7% : *p*<0.05).

Lipoprotein content was expressed as the ratio of LDL to HDL which is a more reliable predictor of atherosclerosis risk; a ratio<3 being considered as normal. The mean LDL/HDL ratio values for males and females in the tea group were respectively 2.64 ± 1.3 and 2.47 ± 1.5 (Table 1). Tea intake induced significant decreases of 16.5% (*p*<0.05) and 16.6% (*p*<0.05) in males and females during the intervention period to reach ratios of 2.20 ± 0.8 and 2.06 ± 0.7 . These values were further decreased (non significant for the male group and significant *p*<0.05 in the female group) during the wash out period.

Plasma total antioxidant capacity

The mean baseline FRAP values in males and females were 1.13 ± 0.6 mmol/L and 1.18 ± 0.7 mmol/L. Plasma FRAP antioxidant capacity for tea treated male volunteers increased markedly (+440% *p*<0.05) and significantly to reach a mean value of 6.1 ± 1.3 mmol/L (Table 1). This value was further significantly increased (+58.8%; *p*<0.01) during the wash out period. Hot water consumption also induced a significant increase of 482% (*p*<0.01) among male controls during the intervention period. Subsequently non significant increases were recorded during

wash out. Similar observations were made for females under tea regimen with a significant 386% increase (*p*<0.05) after 12 weeks. Wash out produced a non significant increase of 59%. A non significant increase was also noted in the female control group during the intervention period. Wash out in this group produced a significant rise (+ 60%, *p*<0.01).

Mean baseline TEAC values were 2.26 ± 0.2 mmol/L in males and 2.43 ± 0.46 mmol/L in females. Tea intake slightly reduced TEAC plasma antioxidant status in men (- 4.1%; *p*<0.05) and in women (- 10.6%; *p*<10.6%) (Table 1). This continuous decrease could be noted throughout the wash out period for males (-6.3%; *p*<0.01) while for females a very slight significant increase was measured. In the control group a decrease in TEAC data was observed for both males (- 15% *p*<0.01) and females (non significant) prior to non significant increases after the wash out.

Discussion

The HPLC-PDA-MS² analysis of the black tea used indicated a highly complex polyphenolic profile with a large distribution of phenolic acids, catechins, flavonols and theaflavins. The total phenols (89 ± 25 mg/g dry mass) compare favorably with the levels found in previously surveyed phenolic rich Mauritian tea infusates (Luximon-Ramma et al., 2005, 2006) and are consistent with those obtained for Chinese Pu'er tea infusates, which ranged from 64 to 126 mg/g (Shao et al., 1995).

The antioxidant capacity of the black tea used in the clinical trial as reflected by its TEAC (1055 ± 25 μmol/g) and FRAP (825 ± 23 μmol/g) values was among the highest when compared to other commercially available Mauritian teas, which ranged from 423 ± 31 μmol/g to 1147 ± 58 μmol/g for TEAC and 357 ± 21 μmol/g to 927 ± 51 μmol/g for TEAC (Luximon-Ramma et al., 2005). Previous studies on the antioxidant power of different teas using the FRAP assay (Benzie and Szeto, 1999) found values of 132–1654 μmol/g for black teas. It is noteworthy that the black tea FRAP data was 52% higher than that of China black tea, 60% higher than that of Pu Li, tea, 38% higher than that of oolong tea (a

semi-fermented tea), 28% higher than that of Jasmine tea and 21% higher than that of Japanese teas.

The daily intake of 9 g of black tea (corresponding to 1.5%; w/v) represented a supplementation of 738 mg of polyphenols/day. This tea dose was consistent with the range of the doses (0.7 to 1.6% w/v.) utilized by Langley-Evans (2000) and Shimada et al. (2004) to study the effects of Oolong, black and green teas on plasma antioxidant capacity and a number of risk factors in patients with coronary artery disease. While some human intervention studies evaluating effects of tea consumption on selective biomarkers of oxidative stress have been confronted with certain confounding lifestyle factors, this study was designed to minimize effects of smoking and alcohol on levels of the measured biomarkers. It is impossible for all subjects to maintain the same food consumption throughout the study period. The subjects were asked to maintain their former diet during the study and it was expected that the randomized design could balance eventual bias between the study and control groups. Nevertheless their daily diet was monitored and diet form data indicated that the fat/lipid content of the population diet did not change significantly during the intervention trial.

The baseline mean values of fasting serum glucose in both normal male and female tea and control groups were higher than the international standard values which vary between 70 and 111 mg/dL. It is noteworthy to situate these data in the context whereby health reports indicate a 23.6% incidence of diabetes in the Mauritian population (age 25–74) and further emphasize that for every known case of diabetes, there was one newly diagnosed case (Ministry of Health and Quality of Life, Mauritius, 2009). Consumption of the black tea infusion for 12 weeks significantly reduced the fasting glucose level by 20% in males and 15% in females. An antihyperglycemic effect of black tea can therefore be anticipated.

In the lipid profiles, circulating triglycerides were reduced and the decrease was more pronounced in men than in women. Triglyceride levels thus fell by 38.6% in males and 28.6% in females in the tea study groups. Relatively marked non-significant reductions were also observed in the control groups thereby questioning the apparent inertness of hot water. Total cholesterol levels were not significantly influenced by tea uptake with slight increases recorded in both intervention and control groups. HDL levels in both genders were significantly increased with tea ingestion and this was reflected in the LDL/HDL ratio, which is increasingly suggested as a better predictor of atherosclerosis risk. This ratio was non-significantly reduced in both male and female tea and control groups. Several studies have evaluated the relationship between tea consumption and cholesterol, triglyceride, LDL and HDL levels. Kono et al. (1992) and Imai and Nakachi (1995) observed in men an inverse relationship of green tea consumption with total cholesterol and triglycerides. The data on the effects of tea intake on triglycerides levels presented in this study are consistent with these findings. In this study black tea consumption did not seem to significantly influence the levels of total and LDL cholesterol.

The data presented demonstrate that an intervention with human subjects, who consumed three cups of black tea per day for a period of 84 days, can significantly improve plasma antioxidant status. Both FRAP assays showed significant increases in the antioxidant status of human plasma at the end of the intervention period for male and female groups. Increase in FRAP values were substantial for women (+ 440%) and men (+ 386%). A high plasma antioxidant level offers protection against oxidative stress through the quenching capacities of endogenously present low molecular weight antioxidants on reactive oxygen species and reactive nitrogen species.

The putative role of polyphenolics as effective *in vitro* antioxidants has been emphasized to explain tea effects. High levels of polyphenolics, including thearubigins and theaflavins in tea can protect cells and tissues from oxidative damage by scavenging oxygen-free radicals.

Tea phenolics may therefore be active antioxidants in the digestive tract and in other tissues after uptake.

In conclusion, moderate intake of black tea improves the levels of independent risk factors of cardiovascular disease and antioxidant defenses in plasma. The effects seem to be ascribed primarily to the synergistic effects of the tea phenolics and most probably with other antioxidant elements (endogenous or exogenous) not characterized in this study. Although the underlying biological mechanisms for these effects and the exact role of phenolics warrant an extensive study, tea may provide an important source of dietary antioxidants in many individuals.

Conflict of interest statement

The authors declare no conflict of interest.

Acknowledgments

This research was fully supported by the Mauritius Research Council. The authors would like to thank the staff of the Cardiac Centre (Pamplemousses), the technicians of the Department of Biosciences, University of Mauritius and Bio Santé Ltd.

References

- Benzie, I.F.F., Strain, J.J., 1996. The ferric reducing ability of plasma (FRAP) as a measure of 'antioxidant power': the FRAP assay. *Anal. Biochem.* 239, 70–76.
- Benzie, I.F.F., Szeto, Y.T., 1999. Total antioxidant capacity of teas by the ferric reducing antioxidant power assay. *J. Agric. Food Chem.* 47, 633–636.
- de Mejia, E.G., Ramirez-Mares, M.V., Puangpraphant, S., 2009. Bioactive components of tea: Cancer, inflammation and behavior. *Brain Behav. Immun.* 23, 721–731.
- Del Rio, D., Stewart, A.J., Mullen, W., et al., 2004. HPLC-MS analysis of phenolic compounds and purine alkaloids in green and black tea. *J. Agric. Food Chem.* 52, 2807–2815.
- Imai, K., Nakachi, K., 1995. Cross sectional study of effects of drinking green tea on cardiovascular and liver disease. *Br. Med. J.* 310, 693–696.
- Kono, S., Shinchi, K., Ikeda, N., et al., 1992. Green tea consumption and serum lipid profiles: a cross-sectional study in Northern Kyushu, Japan. *Prev. Med.* 21, 526–531.
- Lamaison, J.L.C., Carnet, A., 1990. Teneurs en principaux flavonoïdes des fleurs de *Crataegus monogyna* Jacq et de *Crataegus alevigata* (Poiret DC) en fonction de la végétation. *Planta Med. Phytother.* XXV, 12–16.
- Langley-Evans, S.C., 2000. Consumption of black tea elicits an increase in plasma antioxidant potential in humans. *Int. J. Food Sci. Nutr.* 51, 309–315.
- Luximon-Ramma, A., Bahorun, T., Crozier, A., et al., 2005. Characterization of the antioxidant functions of flavonoids and proanthocyanidins in Mauritian black teas. *Food Res. Int.* 38, 357–367.
- Luximon-Ramma, A., Neergheen, V., Bahorun, T., et al., 2006. Assessment of the polyphenolic composition of the organic extracts of Mauritian black teas: a potential contributor to their antioxidant function. *Biofactors* 27, 79–91.
- Ministry of Health and Quality of Life (Mauritius), 2009. The trends in diabetes and cardiovascular disease risk in Mauritius : The Mauritius non communicable diseases survey 2009.
- Pietta, P.G., Simonetti, P., Gardana, C., et al., 1998. Catechin metabolites after intake of green tea infusions. *Biofactors* 8, 111–118.
- Porter, L.J., Hrstich, L.N., Chan, B.C., 1986. The conversion of procyanidins and prodelphinidins to cyanidins and delphinidins. *Phytochemistry* 25, 225–230.
- Rouanet, J.M., Décordé, K., Del Rio, D., et al., 2010. Berry juices, teas, antioxidants and the prevention of atherosclerosis in hamsters. *Food Chem.* 118, 266–271.
- Serafini, M., Ghiselli, A., Ferro-Luzzi, A., 1996. *In vivo* antioxidant effect of green tea and black tea in man. *European J. Clin. Nutr.* 50, 28–32.
- Shao, W., Powell, C., Clifford, M.N., et al., 1995. The analysis by HPLC of green, black and Pu'er teas produced in Yunnan. *J. Sci. Food Agric.* 69, 535–540.
- Sharangi, A.B., 2009. Medicinal and therapeutic potentialities of tea (*Camellia sinensis* L.)—A review. *Food Res. Int.* 42, 529–535.
- Shimada, K., Kawarabayashi, T., Tanaka, A., et al., 2004. Oolong tea increases plasma adiponectin levels and low-density lipoprotein particle size in patients with coronary artery disease. *Diabetes Res. Clin. Pract.* 65, 227–234.
- Singleton, V.L., Rossi, J.A., 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Viticult.* 16, 144–153.
- Stangl, V., Lorenz, M., Stangl, K., 2006. The role of tea and tea flavonoids in cardiovascular health. *Mol. Nutr. Food Res.* 50, 218–228.
- USDA Food Composition database, 2006. <http://www.nal.usda.gov/fnic/foodcomp2006>.
- van het Hof, K.H., de Boer, H.S., Wiseman, S.A., et al., 1997. Consumption of green and black tea does not increase resistance of low-density lipoprotein to oxidation in humans. *Am. J. Clin. Nutr.* 66, 1125–1132.