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Effects of rooibos (*Aspalathus linearis*) on oxidative stress and biochemical parameters in adults at risk for cardiovascular disease

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ABSTRACT

Ethnopharmacological relevance: In South Africa, the plant *Aspalathus linearis* (Brum.f) Dahlg. (Fabaceae) is traditionally used as a "tea" referred to as rooibos or redbush. This plant has been listed as a medicinal plant based mostly on anecdotal evidence.
Aims of the study: Despite a long history of traditional use in South Africa, very little scientific data are available from controlled clinical trials confirming its popular use. The aim of the present study was to investigate the effect of rooibos on biochemical and oxidative stress parameters in adults at risk for cardiovascular disease.
Materials and methods: After a washout period of 2 weeks, 40 volunteers consumed six cups of fermented/traditional rooibos daily for 6 weeks, followed by a control period. Blood biochemical parameters indicative of antioxidant activity and content (total polyphenols), lipid peroxidation (conjugated dienes – CDs, thiobarbituric acid reactive substances – TBARS), redox status (total glutathione – tGSH, ratio of reduced to oxidized glutathione – GSH:GSSG), lipid profile (total cholesterol, low density lipoprotein – LDL and high density lipoprotein – HDL cholesterol and triacylglycerol levels) and liver and kidney function were measured at the end of each study period.
Results: Plasma antioxidant capacity was not altered, but plasma total polyphenol levels increased significantly after rooibos consumption compared with the control levels (from 79.8 ± 16.9 mg/L to 89.8 ± 14.1 mg/L). Significant decreases in plasma markers of lipid peroxidation were found after rooibos consumption, as reported by levels of CDs (167.3 ± 29.5 nmol/mL vs. 108.8 ± 20.1 nmol/mL) and TBARS (1.9 ± 0.6 μmol/L vs. 0.9 ± 0.3 μmol/L). Reduced glutathione (797 ± 238 μmol/L vs. 1082 ± 140 μmol/L) and the GSH:GSSG ratio (41 ± 14 vs. 76 ± 17) were both significantly increased after consumption of rooibos. The lipid profiles showed that rooibos consumption, compared with the control values, significantly decreased serum LDL-cholesterol (4.6 ± 1.3 mmol/L vs. 3.9 ± 0.7 mmol/L) and triacylglycerols (1.7 ± 0.8 mmol/L vs. 1.2 ± 0.7 mmol/L), while HDL-cholesterol (0.9 ± 0.1 mmol/L vs. 1.2 ± 0.2 mmol/L) was significantly increased.
Conclusion: Confirming its popular use, consumption of fermented, traditional rooibos significantly improved the lipid profile as well as redox status, both relevant to heart disease, in adults at risk for developing cardiovascular disease.

Abbreviations: AAE, ascorbic acid equivalents; ABTS, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonate); ANOVA, analysis of variance; ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate transaminase; BMI, body mass index; CDs, conjugated dienes; CHD, coronary heart disease; CHF, coronary heart failure; CPUT, Cape Peninsula University of Technology; CVD, cardiovascular disease; D.Bili, unconjugated bilirubin; EDTA, ethylenediaminetetraacetic acid; FRAP, ferric-reducing ability of plasma; GGT, gamma glutamyl transferase; GSH:GSSG, ratio of reduced to oxidized glutathione; GSSG, oxidized glutathione; HDL-C, high density lipoprotein cholesterol; Hs-CRP, high sensitive C-reactive protein; LDH, lactate dehydrogenase; LDL-C, low density lipoprotein cholesterol; LPO, lipid peroxidation; M2VP, 1-methyl-2-vinylpyridinium trifluoromethanesulphonate; MDA, malondialdehyde; ORAC, oxygen radical absorbance capacity; SD, standard deviation; SST, serum separator tube; TAC, total antioxidant capacity; TBARS, thiobarbituric acid reactive substances; T.Bili, total bilirubin; TE, trolox equivalents; tGSH, total glutathione; USDA, U.S. Department of Agriculture.

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1. Introduction

The effect of rooibos herbal tea consumption on oxidative stress-related diseases, such as cardiovascular disease (CVD), has never been investigated before. Rooibos, a unique South African herbal tea, is made from the leaves and stems of the fynbos plant *Aspalathus linearis* (McKay and Blumberg, 2007; Joubert et al., 2008; Marnewick, 2009). Rooibos is naturally caffeine free and contains very low levels of tannins (Blommaert and Steenkamp, 1978; Galasko et al., 1989). Since its first traditional use in the 1700s, rooibos has been linked to numerous health promoting properties, but substantiation of these health claims are scarce. The popularity of rooibos as a health beverage is not only growing locally but also internationally. Rooibos is an important dietary source of antioxidants containing mostly flavonoids, but also the unique C–C linked dihydrochalcone glucoside, aspalathin (Koeppen and Roux, 1965) and the recently discovered cyclic dihydrochalcone, aspalalinin (Shimamura et al., 2006). Numerous studies have reported on the in vitro antioxidant activity of rooibos, using various types of extracts of rooibos in a number of different assay systems (Yoshikawa et al., 1990; Ito et al., 1991; Lamosova et al., 1997; Von Gadow et al., 1997a,b; Joubert et al., 2004), as well as in vivo activity in experimental rats (Marnewick et al., 2003). Rooibos has been shown to be antimutagenic (Marnewick et al., 2000, 2004), cancer modulating (Marnewick et al., 2005, 2009) and to regenerate coenzyme Q10 with the resultant inhibition of lipid peroxidation in rat liver (Kucharska et al., 2004). More recently, an 8-week randomized placebo-controlled intervention study reported on the antioxidant status of lead factory workers after drinking traditional, fermented rooibos (Nikolova et al., 2007). Modulation of the redox status of these workers was shown by a decreased level of lipid peroxidation [measured as malondialdehyde (MDA) in the plasma] and an increased level of blood glutathione (GSH).

Observational studies linking high dietary intake of plant foods and beverages with a lower incidence of CVD and other chronic diseases suggest the association may be attributed to the polyphenolic antioxidants in these foods (Keli et al., 1996; Knekt et al., 2002; Vita, 2003; Wilcox et al., 2004). To date very little clinical data about the effects of rooibos consumption is available, while no clinical studies have been published on the effects of rooibos on oxidative stress associated with CVD risk. Based on previous in vitro and animal studies, the aim of the present study was to gain further insight into the possible health benefits of rooibos to humans and reports on the modulation of oxidative stress in a population at risk for developing CVD. The rationale for the choice of study population was based on the view that elevated oxidative stress may be required for clear detection of improvements from dietary antioxidant intervention. Hypertension, dyslipidemia and other risk factors for CVD are accepted to be conditions accompanied by elevated oxidative stress (Kaliora and Dedoussis, 2007). Additionally, the safety and possible anti-nutritive effects of beverages are always of interest; thus, assessing whether rooibos consumption within the suggested tea intake range (Elhatton, 2002) has undesirable effects in human subjects was the secondary objective of this study.

2. Materials and methods

2.1. Participants

Potential participants were recruited from the City of Cape Town Metropolitan Municipality of South Africa (SA) through advertisement. Males and females ($n = 83$) interested in the study attended a screening session where fasting blood samples, health and demographic information through self-administered questionnaires and anthropometry were obtained. Inclusion criteria were: (a) healthy adult persons with a stable body weight for 6 months prior to the study, (b) females not pregnant or lactating, (c) conventional diets without undesirable alcohol consumption (>2 drinks per day), (d) absence of CVD, diabetes, renal, hepatic and endocrine disorders, (e) participants not taking any medication, vitamin and/or antioxidant dietary supplements and (f) between 30 and 60 years of age. In addition, recruits were required to have at least two or more of the following risk factors for coronary heart disease (CHD): hypercholesterolaemia (raised cholesterol >5.2 mmol/L), smoking, hypertension (≥140/90 mm Hg) or pre-hypertension (120–139/80–90 mm Hg), increased body mass index (BMI) (25–30 kg/m^2) but not on any medication for these medical conditions. Male and female participants who met the inclusion criteria were entered into the study. None of the participants started any new medications or dietary supplements during the study. The participants' risk for developing heart disease was calculated using the Framingham risk scoring (National Cholesterol Education Programme, 2002). Research guidelines of the Declaration of Helsinki and Tokyo for human studies were followed, while the study protocol was approved by the Faculty of Health and Wellness Sciences Research Ethics Committee of the Cape Peninsula University of Technology (CPUT). All participants gave oral and written informed consent before the study commenced.

2.2. Study design, dietary intake and phytochemical analyses

The controlled clinical trial followed a 12-week pre- and post-measurement single group intervention design. As a placebo for rooibos is not available (rooibos has a very unique taste), a control period where participants consumed an equivalent volume of water was included. Participants first entered a 2-week washout period, where after they consumed six cups of rooibos herbal tea per day for 6 weeks (referred to as the rooibos intervention period) followed by a control period where an equivalent volume (six cups) of water was consumed for 4 weeks. The 4-week period is sufficient to prevent a carry over effect for flavonoids, as most flavonoids have been shown to have short elimination half-lives (Scalbert and Williamson, 2000; Scalbert et al., 2002). During the washout, rooibos intervention and control periods, participants were instructed to follow their habitual diet, but avoiding beverages such as coffee, tea, cocoa drinks, red wine and fruit juices and restricting the daily portion intake of apples, oranges, grapes (black and red) and dark chocolate due to either the high flavonoid content and/or total antioxidant capacity (TAC) to minimize the potential of confounding effects. Fasting (10–12 h) blood samples were collected after the completion of each study period. In order to ensure compliance (seasonal changes during the study period), the study was not randomized. Compliance to the dietary restrictions and rooibos consumption was monitored by means of estimated dietary records completed by the participants for three consecutive days per week for 2 weeks during each study period. These records also served to estimate the average daily energy, macronutrient, vitamin C and total flavonoid intakes of each participant throughout the study. To facilitate the dietary intake recording and estimation of the portion sizes consumed, a recording instruction booklet along with food sketches, a set of household measuring volumes and a ruler were presented to the participants during the dietary record completion training session that occurred before the study commenced. The energy, macronutrient (total fat, saturated fatty acids, cholesterol, carbohydrate, and dietary fiber), and vitamin C intakes were estimated using FoodFinder 3, the nutrient analysis software programme of the South African Medical Research Council (FoodFinder 3, 2002), and the total flavonoid intakes using the United States Department of Agriculture (USDA) database for the flavonoid content of selected foods (USDA Database for the flavonoid content, 2007).

The rooibos used in this study was the fermented, traditional type of superior grade supplied by Rooibos Ltd. (Clanwilliam, SA). All participants were provided a standard recipe on how to prepare the rooibos beverage (one tea bag per 200 mL freshly boiled water, with a brewing time of 5 min) and had the option of adding milk and/or sugar, as this is how it's traditionally consumed. The full amount (six cups) had to be consumed throughout the day. Similarly prepared rooibos was analyzed in the laboratory for its antioxidant activity and total polyphenol/flavonol/flavanol content.

2.3. Blood collection and blood pressure

Fasting (10–12 h) peripheral venous blood samples were collected into one SST tube and one EDTA tube (BD vacutainers, Plymouth, UK) for serum and plasma. Samples were protected from light and transported on ice to the laboratory for immediate processing the same day. Blood samples were centrifuged (1000 × *g*, 10 min, 4 °C) to obtain plasma and serum and stored at −40 °C until analyzed. Whole blood samples for oxidized glutathione (GSSG) analysis were treated with 30 mM of 1-methyl-2-vinylpyridinium trifluoromethanesulphonate, M2VP, purchased from Merck, SA, before storage at −80 °C.

Participants were required to relax for 5 min before three blood pressure readings, (1 min apart) was taken on the same mornings blood was taken. An automatic blood pressure instrument (Rossmax MV701i, Rossmax International, Ltd., Taiwan) was used to record the blood pressure.

2.4. Biochemical analyses

The soluble solid content of the rooibos preparation was determined gravimetrically (10 repetitions) after drying 1-mL aliquots at 60 °C for 72 h. Flavanols in the rooibos beverage were estimated using the 4-dimethylaminocinnamaldehyde spectrophotometric method described by Treutter (1989) with catechin as the standard. Both chemicals were obtained from Sigma–Aldrich (SA). The method used for the estimation of flavonols in the rooibos beverage was previously described by Mazza et al. (1999) using quercetin (Sigma–Aldrich, SA) as the standard. The total polyphenol content of the rooibos beverage and participant's plasma was determined as gallic acid equivalents using the colorimetric Folin–Ciocalteu method described by Singleton and Rossi (1965).

Fasting serum chemistry analytes were measured at the Oxidative Stress Research Centre of CPUT using an automated analyzer (Technicon RA 1000) and enzymatic kits (Kat Medical, RSA). The analytes included glucose, creatinine, iron, aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT), lactate dehydrogenase (LDH), total bilirubin (T.Bili), unconjugated bilirubin (D.Bili), total protein and high sensitive C-reactive protein (hs-CRP). Total cholesterol, high density lipoprotein (HDL-C) cholesterol and triacylglycerol levels were measured using an autoanalyzer, while the low density lipoprotein (LDL-C) cholesterol was calculated using the Friedewald equation, as all participant's triacylglycerol levels were <4.52 mmol/L (Friedewald et al., 1972).

Using trolox, an aqueous analogue of α-tocopherol, as reference antioxidant, the TAC of both rooibos and plasma samples were estimated via the oxygen radical absorbance capacity (ORAC) assay (Ou et al., 2001) and the azinobis(3-ethylbenzothiazoline-6-sulfonate (ABTS) assay (Re et al., 1999). The ferric-reducing ability of plasma (FRAP), which measures the ability to reduce iron from the ferric to ferrous state, was assessed using the method described by Benzie and Strain (1996).

Lipid peroxidation was assessed by measurement of conjugated dienes (CDs) and thiobarbituric acid reactive substances (TBARS). Plasma TBARS were measured according to the method of Yagi (1984) while the method of Recknagel and Glende (1984) was used for the estimation of plasma CDs.

Determination of reduced to oxidized glutathione (GSH:GSSG) ratio in whole blood was carried out using the method described by Asensi et al. (1999).

2.5. Statistical analyses

All assays were done in triplicate or duplicate as specifically outlined. The results are presented as means ± SD. Analysis of variance (ANOVA) was used to determine whether the means of the different study periods differed significantly. When the ANOVA was positive ($P < 0.05$), a Student–Newman–Keuls test for pairwise comparison of the different study periods was performed. Prior to the ANOVA test, the Levene's test for equality of variances was performed and if positive ($P < 0.05$), a logarithmic transformation to the data was done or a non-parametric statistic applied, in this case, the Wilcoxon test. In all analyses, a P value of <0.05 was considered significant.

3. Results

3.1. Phytochemical content and in vitro antioxidant activity of fermented rooibos

The antioxidant capacity and total antioxidant content of the fermented/traditional rooibos herbal tea was determined in the laboratory before the study commenced. The soluble solids mass per cup (200 mL) of freshly prepared fermented rooibos was 1.91 ± 0.16 g, of which the total polyphenols contributed 63.7 ± 5.2 mg, the flavonols 21.2 ± 2.1 mg and flavanols 2.1 ± 0.13 mg. The antioxidant capacity of the freshly brewed rooibos amounted to 1402 ± 44.1 μmol trolox equivalents (TE) as determined by the ORAC assay, 260.2 ± 24.8 μmol ascorbic acid equivalents (AAE) by the FRAP and 173.5 ± 12.8 μmol TE by the ABTS assay.

3.2. Participant profiles

The study had a very low drop out rate, with 43 eligible participants who enrolled in the study and 40 (14 males; 26 females) who completed the study. Three participants (one female and two males) withdrew voluntarily from the study because of personal reasons. The baseline characteristics of the 40 participants with two or more risk factors for coronary heart disease are presented in Table 1.

3.3. Flavonoid, energy, macronutrient and vitamin C intakes

According to the dietary records, participants complied with all the dietary restrictions with the exception of ∼25% of the

Table 1
Participant profile.

Variable	Number
Age (years)	*46.8 ± 9.7
Body mass index (kg/m^2)	*28.4 ± 5.5
Total cholesterol (mmol/L)	*5.7 ± 1.3
Systolic BP (mm Hg)	*135 ± 16
Diastolic BP (mm Hg)	*85 ± 9
Fasting glucose (mmol/L)	*5.3 ± 0.6
Smokers (%)	25
Family history of CVD (%)	40
Framingham score (% risk)	*6 ± 4
Daily total flavonoid intake (mg)	*343 ± 306

BP, blood pressure; CVD, cardiovascular disease.
* Values given as average ± standard deviation.

Table 2
Estimated daily energy, nutrient and flavonoid intakes of participants based on 3-day food recordings for the washout, rooibos intervention and control periods of the study.

Dietary variables	Study periods		
	[R]Washout	[R]Rooibos	[R]Control
Energy (kJ)	8671 ± 2530	8097 ± 2206	8052 ± 2394
Total fat (g)	85.8 ± 32.3	79.1 ± 24.2	82.3 ± 32.0
Energy – total fat (%)	38.4 ± 7.3	37.2 ± 4.9	37.4 ± 6.8
Saturated fatty acids (SFA) (g)	29.2 ± 11.3	26.8 ± 9.3	27.6 ± 10.8
Energy – SFA (%)	12.9 ± 2.5	12.6 ± 2.5	12.7 ± 2.8
Total carbohydrate (g)	213.7 ± 72.9	197.1 ± 58.8	192.5 ± 68.8
Total dietary fiber (g)	20.3 ± 9.1	17.6 ± 7.7	16.3 ± 7.1
Cholesterol (mg)	267.1 ± 113.1	275.1 ± 99.4	255.7 ± 97.4
Vitamin C (mg)	112.7 ± 21.0	88.5 ± 67.1	84.4 ± 67.0
Total flavonoid (mg)	31.3 ± 24.8	343.2 ± 90*	27.4 ± 24.1

Values in columns are means ± SD (n = 40). R, normal diet followed along with restricted dietary flavonoid food and in particular beverage intakes during these periods.
* $P < 0.001$ where significance refers to differences when comparing control period values with rooibos period values.

participants indicating consuming fruit juices (with tropical fruit blend being the most commonly consumed contributing 3.5 mg flavonoids per day) on 1–3 days per week during all study periods. These intakes were analyzed as part of the total dietary flavonoid intake in each study period. Compliancy was also confirmed by a significant ($P < 0.001$) increase in the participant's plasma polyphenol levels after completion of the rooibos intervention when compared to the control (see Section 3.5). The participant average daily total flavonoid intake was significantly ($P < 0.001$) higher during the rooibos intervention period compared to the control period (Table 2). The participant average daily energy, total fat, carbohydrate, dietary fiber, saturated fatty acid, cholesterol and vitamin C intakes for each study period is summarized in Table 2, that also reflects a fat energy contribution profile indicative of CVD risk across the study periods (total fat energy contribution >35% and saturated fatty acid energy contribution >7%) (Linchtenstein et al., 2006). No statistical differences were found in the participant intake of these nutrients, or the fat energy contribution, between the various study periods ($P > 0.05$).

3.4. Biochemical analysis

No adverse effects as a result of consuming six cups of rooibos per day for 6 weeks were reported by the study participants. Although certain liver (AST, ALT, ALP) and kidney (creatinine) function indicators were significantly ($P < 0.05$) increased in participants after completion of the rooibos intervention when compared with the control, the levels of these indicators were still well within the reference range. None of the other liver and kidney function indicators and glucose levels showed any significant changes when comparing the rooibos intervention with the control, and was also within the reference ranges for those indicators (Table 3). The serum iron levels were not adversely affected after completion of the rooibos intervention and control periods. The consumption of rooibos caused a marked decrease of 14.4% in serum glucose levels compared with the control period, although this was not statistically significant. Although the levels of serum unconjugated and total bilirubin in participants consuming rooibos was significantly lower when compared to the control, they were still within reference range. The participant's systolic and diastolic blood pressures also did not differ significantly ($P > 0.05$) after completion of each study period (Table 3).

Changes in the participant serum lipid profiles are shown in Table 4. Their total cholesterol concentration was reduced by 8.6% after completion of the rooibos intervention period, though not significantly ($P > 0.05$). Their serum triacylglycerol and LDL-C levels were significantly decreased ($P < 0.001$), while their HDL-C levels were significantly increased ($P < 0.001$) when comparing the rooibos intervention with the control period (Table 4). There was no significant change in serum hs-CRP, indicating that the consumption of rooibos did not have an anti-inflammatory effect (Table 4).

Table 3
Changes in serum levels of liver and kidney function indicators, glucose and iron as well as blood pressure of participants after completion of the washout, rooibos intervention and control periods of the study.

Analyte	Study periods		
	[R]Washout	[R]Rooibos	[R]Control
Aspartate aminotransferase (U/L)	21.8 ± 7.5	24.2 ± 14.2*	17.8 ± 8.3
Alanine aminotransferase (U/L)	21.8 ± 14.9	18.8 ± 17.2*	12.9 ± 8.3
Gamma glutamyl transferase (U/L)	30.7 ± 15.9	30.8 ± 17.5	28.1 ± 13.7
Alkaline phosphatase (U/L)	82.7 ± 27.8	79.7 ± 25.0*	65.7 ± 20.2
Lactate dehydrogenase (U/L)	149.5 ± 31.7	180.3 ± 44.0	191.0 ± 86.3
Total proteins (g/L)	82.3 ± 22.2	66.6 ± 4.6	65.5 ± 16.0
Urea (mmol/L)	5.0 ± 1.7	4.1 ± 1.1	4.3 ± 1.3
Creatinine (μmol/L)	96.6 ± 22.5	76.6 ± 22.2*	61.6 ± 22.5
Unconjugated (D) bilirubin (μmol/L)	2.9 ± 1.5	3.3 ± 4.0*	5.0 ± 8.5
Total bilirubin (μmol/L)	8.9 ± 5.9	9.3 ± 4.4*	12.25 ± 8.9
Glucose (mmol/L)	5.6 ± 2.7	4.82 ± 1.7	5.6 ± 1.6
Iron (μmol/L)	16.8 ± 4.7	15.7 ± 4.8	13.9 ± 3.1
Systolic blood pressure (mm Hg)	137 ± 21	135 ± 16	139 ± 18
Diastolic blood pressure (mm Hg)	86 ± 12	85 ± 10	86 ± 10

Values in columns are means ± SD (n = 40). R, normal diet followed along with restricted dietary flavonoid food and in particular beverage intakes during these periods.
* $P < 0.05$ where significance refers to differences when comparing control period values with rooibos period values.

3.5. Oxidative status of the participants

The antioxidant content, activity and potential of the blood were measured as total polyphenols, ORAC, FRAP, ABTS and GSH

Table 4
Changes in serum lipoproteins and hs C-reactive protein of participants after completion of the washout, rooibos intervention and control study periods.

Analyte	Study periods		
	[R]Washout	[R]Rooibos	[R]Control
Total cholesterol (mmol/L)	5.3 ± 0.9	5.3 ± 0.9	5.8 ± 1.3
[$]LDL-C (mmol/L)	3.8 ± 0.8	3.9 ± 0.7*	4.6 ± 1.3
HDL-C (mmol/L)	1.3 ± 0.3	1.2 ± 0.2*	0.9 ± 0.2
Triacylglycerol (mmol/L)	1.6 ± 1.3	1.2 ± 0.7*	1.7 ± 0.8
Hs C-reactive protein (mg/dL)	1.2 ± 0.4	1.2 ± 0.4	1.3 ± 0.4

Values in columns are means ± SD (n = 40). R, normal diet followed along with restricted dietary flavonoid food and in particular beverage intakes during these periods.
* $P < 0.001$ where significance refers to differences when comparing control period values with rooibos period values; all participant triacylglycerol levels were <4.52 mmol/L in order to use the Friedewald calculation to determine LDL-C.

Table 5
Changes in serum total polyphenol and antioxidant capacity (ORAC, ABTS, FRAP), plasma conjugated dienes (CDs) and thiobarbituric acid reactive substances (TBARS) and blood glutathione (tGSH, GSSG) levels in participants after completion of the washout, rooibos intervention and control periods of the study.

Biomarker	Study periods		
	[R]Washout	[R]Rooibos	[R]Control
Total polyphenols (mg/L)	72.3 ± 10.2	89.8 ± 14.1*	79.8 ± 16.9
ORAC (μmol TE/L)	1649 ± 408	1409 ± 186	1389 ± 251
ABTS (μmol TE/L)	325 ± 85	347 ± 95	371 ± 105
FRAP (μmol AAE/L)	339 ± 94	350 ± 85	377 ± 88
tGSH (μmol/L)	716 ± 21	1082 ± 140*	797 ± 238
GSSG (μmol/L)	12.0 ± 2.0	13.2 ± 2.1*	19.0 ± 3.9
GSH:GSSG ratio	59.6 ± 24.6	76.3 ± 16.9*	40.7 ± 13.7
TBARS (μmol/L)	1.6 ± 0.7	0.9 ± 0.3*	1.9 ± 0.6
CDs (nmol/mL)	134.7 ± 20.6	108.8 ± 20.1*	167.3 ± 29.5

Values in columns are means ± SD ($n = 40$). R, normal diet followed along with restricted dietary flavonoid food and in particular beverage intakes during these periods.
* $P < 0.001$ where significance refers to differences when comparing control period values with rooibos period values.

(Table 5). Consuming six cups of rooibos for 6 weeks resulted in a significant increase (11.8%, $P < 0.05$) in the plasma total polyphenol content of the participants when compared with their control period content, while consuming rooibos had no significant effect on the antioxidant activity (measured as ORAC, FRAP or ABTS) of their plasma when compared to the control period activity. The antioxidant potential in their whole blood (measured as reduced glutathione – tGSH) increased significantly ($P < 0.001$) after consuming the rooibos when compared to their control potential, while their GSSG was significantly decreased ($P < 0.001$) after completion of the rooibos intervention period compared with the control period. The ratio of GSH:GSSG also increased significantly ($P < 0.001$) after completion of the rooibos intervention period when compared to the control period.

Plasma lipid peroxidation products were determined by measuring the TBARS and CD concentrations (Table 5). The participant plasma levels of both these products were significantly decreased ($P < 0.001$) after completion of the rooibos intervention period compared with the control period. The change in CD concentration observed between the rooibos intervention period and the control period represents a significant 34.9% decrease. A similar trend was observed with regards to the concentration of the participant plasma TBARS, with the level after completion of the rooibos intervention period being significantly lower ($P < 0.001$) when compared with the control period. The intervention with rooibos was associated with a significant 54% decrease in TBARS when compared to the control period.

4. Discussion

Rooibos, prepared from the leaves and stems of *Aspalathus linearis*, is an important source of antioxidants due to its flavonoid content (Joubert et al., 2005). As the phenolic constituents of rooibos differ from that of green and black teas (*Camellia sinensis*), it is important to elucidate the possible health modulating properties of rooibos in humans. This study is the first of its kind reporting on the effects rooibos has on the lipid profile and oxidative stress indicators in humans at risk for developing CVD.

The consumption of six cups of fermented/traditional rooibos per day for 6 weeks did not cause any adverse effects as reported by the study participants and neither were their serum iron levels altered. These results are in line with the findings obtained from two previous human studies (Hesseling et al., 1979; Breet et al., 2005) and an experimental animal study (Marnewick et al., 2003) and serves to establish the safety of short term consumption of rooibos. Recently, a case study was reported by Sinisalo et al. (2010) that describe a 42-year-old woman who had been diagnosed with a low-grade B-cell malignancy, Waldenstrom's macroglobulinemia, in 2004. She exhibited elevated plasma levels of ALT, GGT and ALP on a clinical visit. The patient stated that she had begun to drink 1L rooibos (Forsman Rooibos tea; Aaro Forsman Oy, Vantaa, Finland) per day, 2 weeks before the clinical examination. This case study is the first report suggesting that rooibos may have adverse hepatic effects. The authors stated that in spite of rooibos's excellent safety record, further studies are recommended to resolve this question (Sinisalo et al., 2010). In the current study, average increases in some of the hepatic enzymes were noted, but may not be of clinical importance, as the values are still within the normal reference ranges, however this could be indicative that some of the volunteers had anomalous reactions to rooibos.

Several intervention studies suggest that dietary intervention with a polyphenol-rich beverage in humans can improve the plasma antioxidant capacity and phenolic content (Lotito and Frei, 2006). The rooibos beverage in the present study contained high levels of total polyphenols with proven antioxidant capacity. The significantly higher daily total flavonoid intake during the rooibos intervention period as compared to the control period was not associated with any significant changes in plasma antioxidant capacity when assessed using three methods: FRAP, ABTS and ORAC. Previous studies have also reported no increase in serum antioxidant status (ORAC, FRAP) of participants after the daily consumption of antioxidant-rich black tea, green tea or cranberry juice (Widlansky et al., 2005; Coimbra et al., 2006; Duthie et al., 2006). In the current study, in light of the restricted dietary flavonoid intake, it is possible that while the sustained concentration of polyphenols may have increased, that of other antioxidant components may have decreased resulting in no net increases in antioxidant capacity (such as found with the decreased vitamin C intake in this study). Since all assays for antioxidant capacity have been suggested to lack specificity, their estimates are therefore not likely to indicate any resultant changes in the plasma antioxidant capacity (Lotito and Frei, 2006). In addition, the plasma antioxidant capacity is a fasting measurement and may not represent the active antioxidant pool since the half-lives of all the individual compounds, including polyphenols (varies from 2 h to 11 h) and non-polyphenols, may fluctuate. The 10–12 h fasting period may also have a more pronounced effect on the non-phenolic antioxidants resulting in the antioxidant capacity to remain unchanged or diminished regardless of increased level of polyphenols. Although a number of studies have suggested that tea flavonoids show anti-inflammatory effects that might reduce risk for CVD (Steptoe et al., 2007), results from this study did not show any significant change in the non-specific marker of generalized inflammation, hs C-reactive protein, after consuming rooibos for 6 weeks.

Drinking tea or taking enriched extracts prepared from *Camellia sinensis* has been previously reported to positively modulate the lipid profile of human participants, for example reducing LDL-cholesterol, but not all studies have reported these positive findings (Davies et al., 2003; Maron et al., 2003; Hirano-Ohmori et al., 2005; Nantz et al., 2009). Our results show that the dietary intervention with rooibos modulated not only the serum lipid profile of the participants by significantly decreasing the triacylglycerol and LDL-cholesterol levels and increasing the HDL-cholesterol level, but it also improved the redox status as shown by the increased GSH:GSSG ratio and reduced lipid peroxidation as shown by the significant reduction of the CDs (34.9%) and the TBARS (52%).

The ability of rooibos to increase the GSH:GSSG ratio was previously shown in experimental rats where both rooibos and *Camellia sinensis* teas significantly increased the ratio via a significant reduction of GSSG. However, the mechanisms may differ, since a notable increase in GSH was also seen with rooibos but not with *Camel-*

lia sinensis in that study (Marnewick et al., 2003). Results from the current study concur with this, and also with another human study reporting a significant increase (47.8%) in the levels of GSH (Nikolova et al., 2007). Reduced glutathione is a powerful intracellular antioxidant that plays a vital role in stabilizing various enzymes and can also be considered a good marker for tissue antioxidant capacity (Van Acker et al., 2000; Wang and Jiao, 2000). Several clinical conditions are associated with a decrease in cellular GSH levels that may result in a lowered cellular redox potential (Exner et al., 2000). A recent study by Campolo et al. (2007) also suggested that blood glutathione analyses be included in dietary supplementation trials to assess the thiol redox status especially in chronic heart failure (CHF) patients, as increased free radical production in these patients could result from abnormalities in intracellular GSH cycling, that was associated with increased lipid peroxidation (measured as MDA) in CHF.

The effects of rooibos consumption on the in vivo markers of lipid peroxidation in humans are not well reported in the scientific literature. Previous animal studies have shown that rooibos reduced age-related lipid peroxide accumulation (measured as TBARS) in the brains of rats consuming this herbal tea for 21 months and inhibited MDA formation in rat tissues and liver microsomal preparations (Marnewick et al., 2005; Ulicna et al., 2006). More recently, a clinical study in lead factory workers also showed that consumption of rooibos significantly decreased plasma MDA concentrations (Nikolova et al., 2007). Results from the current study are in agreement with these previous findings as rooibos herbal tea drinking significantly reduced the plasma levels of TBARS and CDs. The effects of other phenolic-rich beverages, especially *Camellia sinensis*, on lipid peroxidation have been studied more extensively and several studies support an inhibitory role for green and black tea. Proposed mechanisms via which this could be achieved include inhibition of lipid absorption and cholesterol synthesis as well as up-regulation of the LDL receptor (Bursill et al., 2007; Koo and Noh, 2007). The present study is supportive of a LDL-cholesterol lowering effect, as rooibos significantly reduced serum levels of LDL-cholesterol and triacylglycerol. Total cholesterol was also lowered, though not significantly, while HDL-cholesterol was significantly higher when compared to the control. It remains to be elucidated whether the reduction in LDL-cholesterol is achieved by up-regulation of the LDL receptor or by other mechanisms. In this study, consuming rooibos not only significantly reduced plasma lipid peroxidation but also significantly lowered serum LDL-cholesterol levels, and therefore a beneficial role of rooibos in the prevention of CHD may be suggested. The observed decrease in plasma lipid peroxidation could also be a reflection of the improved thiol status brought about by the rooibos-induced enhancement of the GSH:GSSG ratio, discussed earlier. The redox state of cells is known to impact profoundly on cellular functions such as the glutathione S-transferase-mediated elimination of electrophilic xenobiotics and some of the end-products of lipid peroxidation (Rebrin et al., 2005).

While the objectives of this study were accomplished, the following limitations should be considered. Because of the 14-week duration of the study, subjects were not randomized. This was done to reduce intergroup confounding (Kendall et al., 2008) which would have been brought about by a change in dietary intake due to seasonal change in the study period. The lack of blinding in this study was necessitated by the difficulty of producing a placebo to match the taste of rooibos and that lacks rooibos flavonoids, as previously mentioned for black tea (Duffy et al., 2001). Despite these limitations, this study provide the first clinical evidence in humans that chronic consumption of rooibos for 6 weeks significantly improved several biomarkers of blood lipid status. In addition, this study also provides supporting evidence that rooibos reduced oxidative stress by significantly decreasing lipid peroxidation and improving the redox status of adults at risk for developing CVD. Furthermore results from this study are optimistic, contributing to our present understanding of the health promoting properties of rooibos and definitely warrants further studies in this field.

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