Journal of Food Research; Vol. 2, No. 2; 2013
ISSN 1927-0887 E-ISSN 1927-0895
Published by Canadian Center of Science and Education

Effectiveness of *Dichrostachys glomerata* Spice Phenolics in Reduction of Oxidative Stress Associated with Obesity and Type 2 Diabetes; a Randomized, Double-Blind Placebo-Controlled Clinical Trial

Dieudonne Kuate[1,2], Anne Pascale Nouemsi Kengne[1,2], William Dakam[1,3], Blanche Cunegonde Omgba Etoundi[1,4], Ghislain Paka Djiokeng[1], Judith Laure Ngondi[1] & Julius Enyong Oben[1]

[1] Department of Biochemistry, Faculty of Science, University of Yaoundé 1, Yaoundé, Cameroon

[2] Department of Biochemistry, Faculty of Sciences, University of Dschang, Cameroon

[3] Department of Biological Sciences, Faculty of Medicine and Pharmaceutical Sciences, University of Douala, Cameroon

[4] Department of Biochemistry, Faculty of Sciences, University of Douala, Cameroon

Correspondence: Julius Oben, Department of Biochemistry, Faculty of Science, PO Box 812, University of Yaoundé 1, Yaoundé, Cameroon. E-mail: juliusoben@hotmail.com

Received: September 12, 2012 Accepted: January 29, 2013 Online Published: February 19, 2013

doi:10.5539/jfr.v2n2p1 URL: http://dx.doi.org/10.5539/jfr.v2n2p1

Abstract

This work evaluated the effect of *Dichrostachys glomerata* on the improvement of antioxidant biomarkers in obesity and type 2 diabetes participants, in an eight-week randomized, double-blind, placebo-controlled design study. The active (400 mg) or placebo formulation were administered twice daily throughout the study period to two normoglycemic and two diabetic obese random groups. Plasma levels of a total of 8 biochemical parameters were taken at the baseline and after 4 and 8 weeks of treatment. No differences in urate variation were observed while the plasma phenolic content as well as the reduced glutathione level, ascorbate concentration, FRAP value and enzymatic antioxidant activities significantly increased with a concomitant reduction of MDA after 8 weeks compared to placebo ($P < 0.01$). On the contrary to urate and ascorbate, plasma polyphenol content correlated well with FRAP level in both treated groups indicating that phenolics from the spice greatly contributed to the antioxidant activity.

Keywords: polyphenolic-rich food, plasma antioxidants, metabolic syndrome, FRAP, uric acid

1. Introduction

Obesity is the central and causal component the metabolic syndrome. Metabolic syndrome or multiple risk factor syndrome which is the coexistence of several risk factors for cardiovascular disease, is a growing medical problem in both developing and industrialized countries (Grundy, Brewer, Cleeman, Smith, & Lenfant, 2004). The increasing trend in obesity worldwide is therefore accompanied by a growing incidence of diabetes (Gregg et al., 2005). This relation is likely to be linked to a number of metabolic impairments and accompanied by oxidative stress disturbances resulting from the presence of excess free radicals (Moreno-Aliaga, Campión, Milagro, Berjón, & Martínez, 2005). In the diabetic condition, oxidative stress impairs glucose uptake in muscle and adipocytes (Maddux et al., 2001) and decreases insulin secretion from pancreatic ß cells thus causing abnormalities in the secretion and action of insulin (Kawahito, Kitahata, & Oshita, 2009). Disturbances of the antioxidant defense systems in diabetes involves an increase in lipid peroxidation, alteration in antioxidant enzymes and impaired glutathione metabolism (Bagri, Ali, Aeri, Bhowmik, & Sultana, 2009). Increased oxidative stress also underlies the pathophysiology of hypertension and atherosclerosis by directly affecting vascular wall cells (Ohara, Peterson, & Harrison, 1993).

Dietary antioxidants including flavonoids, phenolic acids, vitamin E and C as well as carotenoids, are believed to be effective nutrients in the prevention of oxidative stress related chronic diseases (Scalbert, Manach, Morand, Rémésy, & Jiménez, 2005; Vanamala, Kester, Heuberger, & Reddivari, 2012). Clinical trials and epidemiological studies have established an inverse correlation between consumption of flavonoid-rich foods, in

particular fruits and vegetables and lower incidence of cardiovascular disease, ischemic stroke, aging related disorders, cancer, and other chronic diseases (Bosetti et al., 2005; Joshipura et al., 1999). Antioxidant compounds in human foods or supplementary diets have been found to be beneficial against diabetes (Arulmozhi, Veeranjaneyulu, & Bodhankar, 2004). Further, nutritional intervention by means of antioxidant-enriched weight loss dietary supplements could produce protective effects against the redox unbalance thus increasing the health benefits related to weight loss (Parra, Martinez de Morentin, & Martinez, 2005; Crujeiras, Parra, Rodríguez, Martínez de Morenti, & Alfredo, 2006). Significant and possibly relevant effects on total plasma/serum antioxidant capacity have been reported for phenolic-rich foods and beverages, such as wine, tea, different fruits and their derivatives, both in short- and long-term human studies (Crujeiras et al., 2006; Tulipani et al., 2009; Hunter et al., 2012).

Dichrostachys glomerata (*DG*) (Forssk.). Chiov. is a deciduous tree which produces edible fruits and seeds found in Cameroon and many other countries throughout the world. The fruits are dry dehiscent constricted pods which are commonly used as spices in a traditional soup of the western provinces of Cameroon called "Nah po" eaten along with taro (Tchiégang & Mbougueng, 2005). The hypotensive property of the plant was reported more than four decades ago (Roth & Keller, 1963). Studies in our laboratory showed that fruits from this plant had *in vitro* and *in vivo* antioxidant activity along with LDL oxidation inhibiting property (Kuate, Etoundi, Soukontoua, Ngondi, & Oben, 2010). Studies in diabetic rats showed the ability of DG to reduce fasting blood glucose and glycosylated hemoglobin levels in experimental diabetic rats (Kuate, 2010). A recent study indicated that *DG* spice has an effect on the cardiovascular risk factors associated with obesity and type 2 diabetes (Kuate, Etoundi, Soukontoua, Ngondi, & Oben, 2011). In accordance with the above-mentioned preliminary data about obesity, diabetes, oxidative stress and *DG*, this randomized clinical trial was undertaken to evaluate the effect of *DG* spice on the reduction of oxidative stress in obese patients with and without type 2 diabetes.

2. Materials and Methods

2.1 Study Population

Participants for the study were recruited from the city of Yaoundé, Cameroon and its surrounding metro region through radio and print media advertisement. Eligible participants were male or non-pregnant/non-lactacting females, between the ages of 25-65 years with a BMI greater than 30 kg/m^2. Diagnosis of the type 2 obese/diabetic subjects were based on clinical characteristics; i.e., the presence of obesity (BMI > 30 kg/m^2), no history of ketosis or strong family history of diabetes. All patients with type 2 diabetes met the World Health Organization diagnostic criteria of either an abnormal oral glucose tolerance test, or two abnormal fasting blood glucose (>7.0 mmol/L (126 mg/dL)) or the American Diabetic Association criteria of high HbA1c (>7%). Specific exclusion criteria included: diabetes mellitus requiring daily insulin management; pregnancy/lactation; systemic disease and active infection such as HIV/AIDS, active hepatitis or clinical signs of active malignancy within the past 5 years. To standardize and limit the intake of antioxidants during the study, the subjects were asked to refrain from consuming fruits and vegetables or their juices, tea, coffee, any multivitamin supplements, traditional herbs, alcohol or wine for 1 week before taking the supplementation and throughout the study. They were also asked to keep a food diary. The study was approved by the Cameroon National Ethics Board (Approval No. 006/CNE/MP/07). The purpose, nature, and potential risks of the study were explained to all participants, who gave their written informed consent before participation. The study was done in full accordance with the ethical provisions of the World Medical Association Declaration of Helsinki (as amended by the 52nd General Assembly, Edinburgh, Scotland, & October 2000).

2.2 Test Materials

Dried pods of *DG* were bought from the local market in Yaoundé. The pods were ground and encapsulated in individual packets of capsules. The identical-looking placebo and active formulation capsules contained, respectively, 400 mg of maize-based powder consisting of maltodextrin and 400 mg of ground *DG*.

2.3 Study Design/Intervention

The study was a randomized, double-blind, placebo-controlled design for 8 weeks. A total of 92 obese and/or diabetic participants completed the study (20 males and 72 females). The volunteers were randomly allocated to 4 equal groups: 2 normoglycemic obese groups (DichrosOB and PlaceboOB) and 2 type 2 diabetic obese groups (DichrosOB/DB and PlaceboOB/DB). The participants took either one capsule of placebo or one capsule of *Dichrostachys glomerata* (400 mg of *DG*) 30-60 min before lunch and dinner throughout the study period.

2.4 Anthropometric Measurements

Height was measured with a Harpended™ stadiometer. Body weight and percentage body fat, were assessed using a Tanita™ BC-418 Segmental Body Composition Analyzer/Scale that uses bio-electrical impedance analysis for body composition analysis. BMI was calculated as the ratio of weight to the height in metres square.

2.5 Sample Collection

After a 12-hour overnight fast, blood samples were collected into heparinized tubes at baseline and after 4 and 8 weeks of treatment. The plasma obtained from each blood sample (5 ml of blood) was split into multiple 500 µl aliquots and stored at -20°C until it was needed for the measurement of oxidative stress parameters.

2.6 Analytical Methods

2.6.1 Total Antioxidant Power Assay

FRAP (Ferric Reducing Antioxidant Power) was assayed as described by Benzie and Strain (1996). *Lipid peroxidation product as thiobarbituric acid reactive substances* (TBARS): TBARS was based on reaction of malondialdehyde (MDA), one of end products of lipid peroxidation, with thiobarbituric acid (TBA) (Satho, 1978). *Measurement of vitamin C levels:* The concentrations of ascorbic acid in the plasma were measured by the 2,4-dinitrophenylhydrazine (DNPH) method (Roe & Kuether, 1943). *Measurement of total polyphenols*: The total polyphenol content in plasma samples was quantified with a colorimetric assay using Folin-Ciocalteu's phenol reagent (Sigma, St Louis, Mo) according to the method described by Singleton and Rossi (1965).

2.6.2 Measurement of Serum Uric Acid

Uric acid was measured by an enzymatic colorimetric test using a commercially available kit from Chronolab Diagnostic Group and following the manufacturer's instructions. In this assay, uric acid is oxidized by uricase to allantoine and hydrogen peroxide, which, under the influence of peroxidase, oxidizes sulfonated 2,4-dichlorophenol and 4-aminophenazone to form a red quinoneimine compound. The red dye formed is measured at 520 nm, and its quantity is proportional to the uric acid concentration. The working reagent was prepared by dissolving a lyophilized enzyme mixture containing 60 U/L uricase, 660 U/L peroxidase, 200 U/L ascorbate oxidase, and 1 mmol/L 4-aminophenazone, with a phosphate buffer 50 mmol/L, pH 7.4, containing 4 mmol/L 2,4-dichlorophenol. For the assay, 50 µl of the sample or standard (6 mg/dL uric acid solution) was mixed with 2 mL of working reagent, incubated at room temperature for 10 min, and absorbance readings were taken at 520 nm against a reagent blank. Results were calculated by using the following equation and expressed as milligrams per deciliter: Uric acid (mg/dL) = ((Absorbance sample/Absorbance standard) × Standard concentration).

2.6.3 Measurement of the Reduced Glutathione

The plasma reduced glutathione (GSH) was determined by the method of Beutler and Kelly (1963). The technique involves protein precipitation by meta-phosphoric acid and spectrophotometric assay at 412 nm of the yellow derivative obtained by the reaction of the supernatant with 5-5'dithiobis-2-nitrobenzoic acid. The plasma protein was measured by the method of Lowry, Rosebrough, Farr and Randall (1951).

2.6.4 Measurement of Enzymatic Antioxidant Activities

Superoxide dismutase (SOD) activity was measured by the method of Misra and Fridovich (1972). Plasma (0.5 mL) was diluted to 1.0 mL with distilled water, and 250 µl of chilled ethanol and 150 µl of chilled chloroform were added. The mixture was shaken and centrifuged. The supernatant was used for the assay of enzyme activity. To 1.2 mL of the supernatant was added 1.5 mL of 0.1 mol/L carbonate-bicarbonate buffer, pH 10.2, containing 0.2 mmol/L EDTA. The contents were mixed, and the reaction was initiated by adding 200 µl of epinephrine (pH 3.0, 3 mmol/L) to the buffered reaction mixture. The change in optical density per minute was measured at 470 nm. The enzyme activity was expressed as unit per milligram of protein, where 1 U is defined as the enzyme concentration required to inhibit 50% of epinephrine auto-oxidation in 1 min under the assay conditions.

Catalase (CAT) activity was assayed by the method of Sinha (1972). 0.1 mL of Plasma and 1.5 mL of phosphate buffer were added. To this, 0.4 mL of hydrogen peroxide was added and the reaction was arrested after 30 and 60 second by the addition of 2.0 mL dichromate acetic acid reagent. A control was also carried out simultaneously. All the tubes were heated in a boiling water bath for exactly 10 min, cooled and absorbance read at 620 nm. Standards in the range of 2-10 mmoles were taken and processed as the test. The activity of catalase was expressed as µmoles of hydrogen peroxide consumed/min/mg of protein (unit per milligram of protein).

2.7 Statistical Analysis

The data were summarized (mean, standard error of mean) for Week 0 (Initial), Week 4 and Week 8 (final) and for the intra-group variation and analyzed by SPSS 16.0 for Windows (SPSS Inc., Chicago, Illinois, USA). Because the repeated measurements on each participant were correlated in nature (covariance), a mixed model approach, a flexible tool for analyzing repeated and longitudinal treatments was used to characterize variation between patients and within patients. Statistical analyses were performed on monthly set (same time point) of data in order to investigate the differences between groups, as well as the differences in variables change over time between groups. The interaction term between time and intervention was tested at the 0.05 level of significance. If the interaction was significant, comparisons were made between the treated and placebo groups for each month. Between-treatment changes were tested using 1-way analysis of covariance (ANCOVA), adjusted for baseline values, with the initial value as a covariate. Bilateral correlations between variables were examined using Pearson's correlation coefficients. P values of 0.05 were considered to be the level of statistical significance. P values less than 0.01 and 0.05 are presented in the tables.

3. Results

At the beginning of the study, the groups were similar based upon age, weight, fasting blood glucose, HbA1c (treatment vs placebo). Body weight and other anthropometric and hemodynamic parameters were reduced by the consumption of *DG*. Beneficial changes were observed in blood glucose and lipid levels in these participants (Kuate et al., 2011). Tables 1-4 show the oxidative stress parameters before and after the administration of DG or placebo. *DG* was effective in reducing lipid peroxidation and increasing the total antioxidant capacity of the plasma, glutathione level, plasma polyphenol, ascorbate concentration and enzymatic antioxidant activities both in obese and obese type 2 diabetics (to a greater extent) patients after 8 weeks of treatment ($P < 0.01$). The activities of SOD and catalase were increased by 41.41 and 29.55 % respectively in the obese diabetic group, while urate concentration was not affected in both groups. Correlation was neither observed between FRAP and vitamin C nor FRAP and urate. Indeed, a positive and significant correlation was observed between polyphenol content of the plasma and FRAP value ($r = 0.527$ and 0.806 for OB and OB/DB respectively, $P < 0.01$), between FRAP and GSH level. Consequently, FRAP and MDA levels on one hand and polyphenol and MDA concentrations on the other hand also showed strong inverse correlation ($P < 0.01$) (Tables 5 and 6). Such correlations were not present in the placebo groups.

Table 1. The effect of *DG* on plasma total antioxidant capacity and non-enzymatic antioxidants in obese (OB). Volunteers

Variables	Groups	T0	T4	T8	Change from Baseline T8-T0
FRAP (μM of Cat eq/L)	DG	174.22± 1.64	180.04± 1.33[b]	185.65± 1.14[b]	11.43± 1.33 (+6.7) [†]
	Placebo	177.09± 1.59	176.91± 1.62	177.65± 1.35	0.57± 0.9 (+0.37)
Polyphenol (mg of Cat q/L)	DG	7.12± 0.11	8.81± 0.30[b]	10.43± 0.36[b]	3.32± 0.39 (+47.51) [†]
	Placebo	7.04± 0.07	6.99± 0.28	7.2± 0.25	0.16± 0.26 (+2.57)
Urate (mg/dL)	DG	4.77± 0.07	4.8± 0.07	4.76± 0.06	-0.01± 0.04 (-0.09)
	Placebo	4.81± 0.08	4.76± 0.08	4.82± 0.07	0.01± 0.04 (+0.37)
Ascorbate (mg /L)	DG	10.83± 0.13	10.91± 0.14[a]	11.04± 0.12[b]	0.21± 0.06 (+2.04) [†]
	Placebo	10.69± 0.14	10.66± 0.13	10.71± 0.14	0.01± 0.03 (+0.13)
GSH (μmole/g of protein)	DG	0.91± 0.03	1.03± 0.03[b]	1.16± 0.03[b]	0.25± 0.02 (+27.89)[†]
	Placebo	0.92± 0.02	0.93± 0.02	0.93± 0.02	0.00± 0.01 (+0.25)

[a] $p < 0.05$; compared with Placebo; adjusted for baseline;

[b] $p < 0.01$; compared with Placebo; adjusted for baseline;

[†] $p < 0.05$; compared with Initial; intragroup analysis.

Table 2. The effect of *DG* on plasma total antioxidant capacity and non-enzymatic antioxidants in diabetic (OB/DB) volunteers

Variables	Groups	T0	T4	T8	Change from Baseline T8-T0
FRAP (μM of Cat eq/L)	DG	148.22± 148.22	176.13± 1.55[b]	186.43± 1.68[b]	38.22± 2.39 (+26.10)[†]
	Placebo	147.70±2.06	144.87± 3.82	146.3±2.31	-1.39± 1.52 (-1.03)
Polyphenol (mg of Cat eq/L)	DG	6.86± 0.12	8.91± 0.31[b]	10.41± 0.36[b]	3.54± 0.35 (+52.02) [†]
	Placebo	6.64± 0.12	6.24± 0.24	6.44± 0.26	-0.2± 0.22 (-3.12)
Urate (mg/dL)	DG	5.3± 0.05	5.28± 0.06	5.32± 0.05	0.03± 0.04 (+0.53)
	Placebo	4.98± 0.07	4.95± 0.09	4.99± 0.07	0.01± 0.07 (+0.46)
Ascorbate (mg /L)	DG	7.71± 0.13	7.89± 0.13[a]	8.05± 0.14[b]	0.33± 0.05 (+4.32) [†]
	Placebo	7.52± 0.17	7.56± 0.17	7.54± 0.16	0.02± 0.07 (+0.37)
GSH (μmole/g of protein)	DG	0.79± 0.02	1.03± 0.02[b]	1.07± 0.02[b]	0.29± 0.03 (+38.11)[†]
	Placebo	0.74±0.02	0.73± 0.02	0.74±0.03	-0.01± 0.02 (-0.59)

[a]$p < 0.05$; compared with Placebo; adjusted for baseline;

[b]$p < 0.01$; compared with Placebo; adjusted for baseline;

[†]$p < 0.05$; compared with Initial; intragroup analysis.

Table 3. The effect of *DG* on plasma malondialdehyde and enzymatic antioxidants in obese (OB). volunteers

Variables	Groups	T0	T4	T8	Change from Baseline T8-T0
SOD (U/mg of protein)	DG	1.44± 0.03	1.46± 0.02[b]	1.71± 0.05[b]	0.27± 0.05 (+19.25) [†]
	Placebo	1.47± 1.68	1.46± 0.02	1.48± 0.03	0.01± 0.01 (+0.51)
MDA (μM)	DG	1.47± 0.03	1.22± 0.02[b]	1.14± 0.02[b]	-0.33± 0.02 (-22.07)[†]
	Placebo	1.48± 0.02	1.47± 0.02	1.44± 0.02	-0.03± 0.01 (-2.27)
CAT (U/mg of protein)	DG	1.96± 0.02	2.13± 0.03[b]	2.27± 0.05[b]	0.3± 0.04 (+15.61)[†]
	Placebo	2.02± 0.03	2.01± 0.02	2.05± 0.02	0.03± 0.02 (+1.6)

[b]$p < 0.01$; compared with Placebo; adjusted for baseline;

[†]$p < 0.05$; compared with Initial; intragroup analysis.

Table 4. The effect of *DG* on plasma malondialdehyde and enzymatic antioxidants in diabetic (OB/DB) volunteers

Variables	Groups	T0	T4	T8	Change from Baseline T8-T0
SOD (U/mg of protein)	DG	1.13± 0.03	1.37± 0.03 [b]	1.57± 0.04[b]	0.44± 0.04 (+41.41)[†]
	Placebo	1.14±0.03	1.16± 0.02	1.13±0.03	-0.06± 0.05 (-4.72)
MDA (μM)	DG	2.11± 0.08	1.50± 0.06[b]	1.19± 0.05[b]	-0.92± 0.11 (-40.53)[†]
	Placebo	2.21±0.08	2.17± 0.07	2.27±0.06	0.06± 0.03 (+3.75)
CAT (U/mg of protein)	DG	1.66± 0.04	1.91± 0.03[b]	2.13±0.04[b]	0.47± 0.04 (+29.55)[†]
	Placebo	1.69±0.03	1.66± 0.05	1.66±0.06	-0.03± 0.06 (-1.8)

[b]$p < 0.01$; compared with Placebo; adjusted for baseline;

[†]$p < 0.05$; compared with Initial; intragroup analysis.

Table 5. Correlations between plasma non-enzymatic antioxidant parameters in obese (OB) volunteers

		Frap	PolyP	Urate	VitC	MDA	GSH
Frap	Pearson Correlation	1	0.527**	0.077	0.080	-0.614**	0.307*
	Sig. (2-tailed)		0.000	0.530	0.516	0.000	0.010
PolyP	Pearson Correlation	0.527**	1	-0.012	-0.053	-0.518**	0.425**
	Sig. (2-tailed)	0.000		0.925	0.668	0.000	0.000
Urate	Pearson Correlation	0.077	-0.012	1	-0.114	-0.152	-0.235
	Sig. (2-tailed)	0.530	0.925		0.352	0.212	0.052
VitC	Pearson Correlation	0.080	-0.053	-0.114	1	-0.024	0.145
	Sig. (2-tailed)	0.516	0.668	0.352		0.847	0.235
MDA	Pearson Correlation	-0.614**	-0.518**	-0.152	-0.024	1	-0.273*
	Sig. (2-tailed)	0.000	0.000	0.212	0.847		0.023
GSH	Pearson Correlation	0.307*	0.425**	-0.235	0.145	-0.273*	1
	Sig. (2-tailed)	0.010	0.000	0.052	0.235	0.023	

**. Correlation is significant at the 0.01 level (2-tailed);

*. Correlation is significant at the 0.05 level (2-tailed).

Table 6. Correlations between plasma non-enzymatic antioxidant parameters in diabetic (OBDB) volunteers

		Frap	PolyP	Urate	VitC	MDA	GSH
Frap	Pearson Correlation	1	0.806**	0.082	0.176	-0.802**	0.839**
	Sig. (2-tailed)		0.000	0.500	0.149	0.000	0.000
PolyP	Pearson Correlation	0.806**	1	-0.132	0.237*	-0.597**	0.698**
	Sig. (2-tailed)	0.000		0.279	0.050	0.000	0.000
Urate	Pearson Correlation	0.082	-0.132	1	-0.008	-0.098	-0.048
	Sig. (2-tailed)	0.500	0.279		0.947	0.422	0.696
VitC	Pearson Correlation	0.176	0.237*	-0.008	1	-0.070	0.167
	Sig. (2-tailed)	0.149	0.050	0.947		0.570	0.170
MDA	Pearson Correlation	-.802**	-0.597**	-0.098	-0.070	1	-0.591**
	Sig. (2-tailed)	0.000	0.000	0.422	0.570		0.000
GSH	Pearson Correlation	0.839**	0.698**	-0.048	0.167	-0.591**	1
	Sig. (2-tailed)	0.000	0.000	0.696	0.170	0.000	

*. Correlation is significant at the 0.05 level (2-tailed);

**. Correlation is significant at the 0.01 level (2-tailed)

4. Discussion

To recap, we investigated the long-term effect of *DG* supplements on plasma antioxidant capacity in normoglycemic and type 2 diabetic obese subjects. A large, statistically significant increase in plasma antioxidant capacity as assessed by FRAP was observed. This was associated with a concomitant reduction of body weight, BMI, waist and hip circumference, body fat, blood pressure, blood cholesterol, triglycerides, glucose, and glycosylated hemoglobulin published elsewhere (Kuate et al., 2011). Ferric reducing antioxidant power is a measure of total antioxidant protection in plasma or serum and is generally correlated with uric acid concentrations and, to a lesser extent, with ascorbic acid concentrations Benzie and Strain (1996). For instance, fructose in consumed apples was recently shown to elevate plasma uric acid, and this correlated with the

measured plasma antioxidant activity increase (Lotito & Frei, 2004). In the literature, the major contributors to FRAP values are uric acid (60%) and vitamin C (15%), followed by protein (10%), bilirubin (5%), and a-tocopherol (5%). Other antioxidants, including plasma/serum phenolic acids and polyphenolic compounds, have been thought to contribute about 5% of the FRAP values Benzie and Strain (1996). For this reason, the contribution of polyphenols to FRAP could be considered negligible. Our results are not in agreement with this premise as plasma uric acid variation did not parallel change in FRAP values after 4 and 8 weeks of *DG* supplementation. The result of the interventional study was that *DG* exerts an antioxidant effect via its polyphenolic content. Previous studies have shown that circulating MDA levels are higher in diabetic and obese subjects than in non-obese healthy controls (Prazny, Skrha, & Hilgertova, 1999). A reduction of fasting blood glucose levels as well as MDA levels have been previously reported to accompany weight loss in obese subjects (Yesilbursa, Serdar, Sarac, & Jale, 2005). The decrease in plasma MDA concentration observed in the current trial suggests that the whole process of lipid peroxidation was diminished by regular consumption of *DG*. Different studies have shown that hyperglycemia and hypercholesterolaemia associated obesity diminishes the antioxidant defense system and decreases the activity of SOD and CAT, thereby elevating the lipid peroxide content (Anila & Vijayalakshmi, 2003). The antioxidant enzyme SOD plays a pivotal role in oxygen defense metabolism by intercepting and reducing superoxide to water and molecular oxygen (Lin, Tsai, Lee, & Chang, 2005). The decrease in SOD activity in diabetic patients results from inactivation by hydrogen peroxide or by glycosylation of the enzyme, which have been reported to occur in diabetes (Ravi et al., 2004). CAT is involved in the reduction of hydrogen peroxide. Decrease in CAT activity could result from inactivation by superoxide radical and glycation of the enzyme. This may account for lower SOD and CAT activities in the plasma of diabetic patients. Following treatment with DG for 2 months, the activities of both SOD and CAT was augmented in obese and type 2 diabetic patients which could be attributed to the strong antioxidant property of the said spice. GSH is a reactive non-protein thiol in living organisms, which performs a key role in coordinating innate antioxidant defense mechanisms. It is involved in the maintenance of normal structure and function of cells, probably through its redox and detoxification reactions (Gueeri, 1995). Thus, the decrease in plasma GSH level in diabetic patients might initiate a set of reactions with the generated free radicals which are largely consumed by GSH-related enzymes. In our study, a rise in GSH level was observed in the *DG*-treated patients in both groups. This probably indicates that *DG* can either increase the biosynthesis of GSH or reduce the extent of oxidative stress leading to less GSH degradation, or it may have both effects.

Nutritional guidelines (World Health Organization, 1990) suggest that an increase in the consumption of foods rich in antioxidant nutrients may decrease or prevent the risk of many diseases caused by oxidative stress. Therefore, increasing the plasma/serum antioxidant status has been proposed as a method for preventing the development of cancer, cardiovascular disease, diabetes, and other diseases (Ruiz-Roso, Quintela, De la Fuente, Haya, & Pérez-Olleros, 2010). Phytochemicals, especially the phenolics found in fruits and vegetables, have been proposed as the major bioactive compounds providing the health benefits associated with diets rich in plant-foods. Polyphenols are efficient scavengers of free radicals and breakers of lipid peroxidative chain reactions (Teissedre & Landrault, 2000). Besides this well known antioxidant activity, polyphenols also have hypoglycemic and anti-inflammatory effects and may protect low-density lipoproteins (LDL) from oxidative modification (Kuo, Leavitt, & Lin, 1998). Some of those activities were observed in a previous *in vitro* study of the antioxidant property of *DG* (Kuate et al., 2010).

Several studies have reported increases in antioxidant capacity of plasma following consumption of antioxidant-rich fruit, vegetables, teas, wines and grape juice (Benzie, Szeto, Strain, & Tomlinson, 1999). This effect has been ascribed to the low molecular weight phenolics in many plant-based foods which can act as antioxidants because their extensive conjugated p-electron systems allow ready donation of electrons or hydrogens from the hydroxyl moieties to free radicals (Scott, 1997).

Direct evidence that polyphenols are absorbed by humans is provided by Maiani, Serafini, Salucci, Azzini and Ferro-Luzzi (1997), who showed the appearance of epigallocatechingallate in plasma 30 min after the ingestion of 300 mL of green tea. Our results show that plasma polyphenols increase after supplementation with *DG*, suggesting that the absorption of polyphenols might occur, mainly in the gastro duodenal region and, in lesser amounts, in the jejunum. It has to be taken into account, that *DG* phenolic compounds, which may be absorbed from the gastrointestinal tract, would contribute to the plasma antioxidant capacity and simultaneously affect FRAP values (Pignatelli et al., 2006). Another plausible mechanism for polyphenols-induced antioxidant action is focused on the gastrointestinal tract. A part of polyphenols are instead localized in the gastrointestinal tract, and never reach blood. They can scavenge free radicals locally in the stomach, preventing lipid peroxidation and, at the same time, spare other antioxidants from oxidation. By this way, although acting locally, the polyphenols

could influence the whole organism and the plasma concentration of various antioxidants, which in turn, could affect plasma FRAP values (Kanner & Lapidot, 2001).

5. Conclusion

In conclusion consumption of *DG* by normoglycemic obese and type 2 diabetic volunteers resulted in an improvement of oxidative stress in obese and type 2 diabetic patients. This effect could be related to its high polyphenol content. Consumption of antioxidant substances contained in this fruit could be a useful strategy in the design of dietary supplement, and along with the weight and blood lipid and glucose reduction observed, could contribute to the improvement of cardiovascular risk factors related to obesity and type 2 diabetes. Further study will be undertaken to assess if DG treatment is associated with amelioration of artery dilatation that is usually reduced in both diabetic and obese patients.

References

Anila, L., & Vijayalakshmi, N. R. (2003). Antioxidant action of flavonoids from *Magnifiers Indica* and *Emblica Officinalis* in hypercholesterolaemic rats. *Food Chemistry, 83*, 569-574. http://dx.doi.org/10.1016/S0308-8146(03)00155-9

Arulmozhi, D. K., Veeranjaneyulu, A., & Bodhankar, S. L. (2004). Neonatal streptozotocin-induced rat model of Type 2 diabetes mellitus: a glance. *Indian Journal of Pharmacology, 36*, 217-221.

Bagri, P., Ali, M., Aeri, V., Bhowmik, M., & Sultana, S. (2009). Antidiabetic effect of *Punica granatum* flowers: effect on hyperlipidemia, pancreatic cells lipid peroxidation and antioxidant enzymes in experimental diabetes. *Food Chemistry and Toxicology, 47*, 50-54. http://dx.doi.org/10.1016/j.fct.2008.09.058

Benzie, I. F, Szeto, Y. T, Strain, J. J, & Tomlinson, B. (1999). Consumption of green tea causes rapid increase in plasma antioxidant power in humans. *Nutrition and Cancer, 34*, 83-87. http://dx.doi.org/10.1207/S15327914NC340112

Benzie, I. F. F., & Strain, J. J. (1996). The ferric reducing ability of plasma FRAP. as a measure of "antioxidant power": the FRAP assay. *Analytical Biochemistry, 239*, 70-76. http://dx.doi.org/10.1006/abio.1996.0292

Beutler, E., & Kelly, B. M. (1963). The effect of sodium nitrite on red cell GSH. *Experientia, 19*, 96-97. http://dx.doi.org/10.1007/BF02148042

Bosetti, C., Spertini, L., Parpinel, M., Gnagnarella, P., Lagiou, P., Negri, E., … La Vecchia. (2005). Flavonoids and breast cancer risk in Italy. *Cancer Epidemiology, Biomarkers & Prevention, 14*, 805-808. http://dx.doi.org/10.1158/1055-9965.EPI-04-0838

Crujeiras, A. B., Parra, M. D., Rodríguez, M. C., Martínez de Morenti, B. E., & Alfredo Martínez, J. (2006). A role for fruit content in energy-restricted diets in improving antioxidant status in obese women during weight loss. *Nutrition, 22*, 593-599. http://dx.doi.org/10.1016/j.nut.2006.03.008

Gregg, E. W., Cheng, Y. J., Cadwell, B. L., Imperatore, G., Williams, D. E., Flegal, K. M., … Williamson, D. F. (2005). Secular trends in cardiovascular disease risk factors according to body mass index in US adults. *Journal of the American Medical Association, 293*, 1868-1874. http://dx.doi.org/10.1001/jama.293.15.1868

Grundy, S. M., Brewer, H. B., Cleeman, J. I., Smith, S. C., & Lenfant, D. (2004). Definition of metabolic syndrome: Report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition. *Circulation, 109*, 433-438. http://dx.doi.org/10.1161/01.CIR.0000111245.75752.C6

Gueeri, H. (1995). Influence on prolonged ethanol intake on the level and turnover of alcohol and aldehyde dehydrogenase and glutathione. *Advances in Experimental Medicine and Biology, 23*, 133-134.

Hunter, D. C., Brown, R., Green, T., Thomson, C., Skeaff, M., Williams, S., … Skinner, M. A. (2012). Changes in markers of inflammation, antioxidant capacity and oxidative stress in smokers following consumption of milk, and milk supplemented with fruit and vegetable extracts and vitamin C. *International Journal of Food Sciences and Nutrition, 631*, 90-102. http://dx.doi.org/10.3109/09637486.2011.601286

Joshipura, K. J., Ascherio, A., Manson, J. E., Stampfer, M. J, Rimm, E. B., Speizer, F. E., … Willett, W. C. (1999). Fruit and vegetable intake in relation to risk of ischemic stroke. *Journal of the American Medical Association, 282*, 1233-1239. http://dx.doi.org/10.1001/jama.282.13.1233

Kanner, J., & Lapidot, T. (2001). The stomach as a bioreactor: dietary lipid peroxidation in the gastric fluid and the effects of plant-derived antioxidants. *Free Radical Biology and Medicine, 31*, 1388-1395. http://dx.doi.org/10.1016/S0891-5849(01)00718-3

Kawahito, S., Kitahata, H., & Oshita, S. (2009). Problems associated with glucose toxicity: Role of hyperglycemia-induced oxidative stress. *World Journal of Gastroenterology, 15*, 4137-4142. http://dx.doi.org/10.3748/wjg.15.4137

Kuate, D. (2010). *Effects of some spices on glucose and lipid metabolism and oxidative stress* (PhD thesis). University of Yaounde I.

Kuate, D., Etoundi, B. C. O., Soukontoua, Y. B, Ngondi, J. L., & Oben, J. E. (2010). Antioxidant characteristics of *Dichrostachys glomerata* spice extracts. *CyTA - Journal of Food,* 23-37. http://dx.doi.org/10.1080/19476330903129126

Kuate, D., Etoundi, B. C. O., Soukontoua, Y. B., Ngondi, J. L., & Oben, J. E. (2011). Effects of *Dichrostachys glomerata* spice on cardiovascular diseases risk factors in normoglycemic and type 2 diabetic obese volunteers. *Food Research International, 44*, 1197-1202. http://dx.doi.org/10.1016/j.foodres.2010.09.037

Kuo, S. M., Leavitt, P. S., & Lin, C. P. (1998). Dietary flavonoids interact with trace metals and affect metallothionein level in human intestinal cells. *Biological Trace Element Research, 62*, 135-153. http://dx.doi.org/10.1007/BF02783967

Lin, Y. F., Tsai, H. L., Lee, Y. C., & Chang, S. J. (2005). Maternal vitamin E supplementation affects the antioxidant capability and oxidative status of hatching chicks. *Journal of Nutrition, 135*, 2457-2461.

Lotito, S. B., & Frei, B. (2004). The increase in human plasma antioxidant capacity after apple consumption is due to the metabolic effect of fructose on urate, not apple-derived antioxidant flavonoids. *Free Radical Biology and Medicine, 37*, 251-258. http://dx.doi.org/10.1016/j.freeradbiomed.2004.04.019

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with Folin's phenol reagent. *Journal of Biological Chemistry, 193*, 265-275.

Maddux, B. A., See, W., Lawrence, Jr J. C., Goldfine, A. L., Goldfine, I. D., & Evans, J. L. (2001). Protection against oxidative stress-induced insulin resistance in rat L6 muscle cells by micromolar concentrations of alpha-lipoic acid. *Diabetes, 50*, 404-410. http://dx.doi.org/10.2337/diabetes.50.2.404

Maiani, G., Serafini, M., Salucci, M., Azzini, E., & Ferro-Luzzi, A. (1997). Application of a new high-performance liquid chromatographic method for measuring selected polyphenols in human plasma. *Journal of Chromatography B: Biomedical Sciences and Applications, 692*, 311-317. http://dx.doi.org/10.1016/S0378-4347(97)00009-1

Misra, H. P., & Fridovich, I. (1972). The role of superoxide anion in the autooxidation of epinephrine and a simple assay for superoxide dismutase. *Journal of Biological Chemistry, 247*, 3170-3175.

Moreno-Aliaga, M. J., Campión, J., Milagro, F. I., Berjón, A., & Martínez, J. A. (2005). Adiposity and Proinflammatory state: the chicken or the egg. *Adipocytes, 1*, 1-16.

Ohara, Y., Peterson, T. E., & Harrison, D. G. (1993). Hypercholesterolemia increases endothelial superoxide anion production. *Journal of Clinical Investigation, 91*, 2546-2551. http://dx.doi.org/10.1172/JCI116491

Parra, M. D., Martinez de Morentin, B. E., & Martinez, J. A. (2005). Postprandial insulin response and mitochondrial oxidation in obese men nutritionally treated to lose weight. *European Journal of Clinical Nutrition, 59*, 334-340. http://dx.doi.org/10.1038/sj.ejcn.1602078

Pignatelli, P., Ghiselli, A., Buchetti, B., Carnevale, R., Natella, F., Germano, G., … Violi, F. (2006). Polyphenols synergistically inhibit oxidative stress in subjects given red and white wine. *Atherosclerosis, 188*, 77-83. http://dx.doi.org/10.1016/j.atherosclerosis.2005.10.025

Prazny, M., Skrha, J., & Hilgertova, J. (1999). Plasma malondialdehyde and obesity: is there a relationship? *Clinical Chemistry and Laboratory Medicine, 37*, 1129-1130. http://dx.doi.org/10.1515/CCLM.1999.164

Ravi, K., Ramachandran, B., & Subramanian, S. (2004). Protective effect of *Eugenia jambolaa* seed kernel on tissue antioxidants in streptozotocin-induced diabetic rats. *Biological & Pharmaceutical Bulletin, 7*, 1212-1217. http://dx.doi.org/10.1248/bpb.27.1212

Roe, J. H., & Kuether, C. A. (1943). The determination of ascorbic acid in the whole blood and urine through the 2,4-dinitrophenylhydrazine derivative of dehydroascorbic acid. *Journal of Biological Chemistry, 147*, 399-407.

Roth, L. W., & Keller, F. (1963). *Dichrostachys glomerata* hypotensive agent. US Patent 3, 089, 817.

Ruiz-Roso, B., Quintela, J. C., De la Fuente, E., Haya, J., & Pérez-Olleros, L. (2010). Insoluble Carob Fiber

Rich in Polyphenols Lowers Total and LDL Cholesterol in Hypercholesterolemic Sujects. *Plant Foods for Human Nutrition, 65*, 50-56. http://dx.doi.org/10.1007/s11130-009-0153-9

Satho, K. (1978). Serum lipid peroxidation in cerebrovascular disorders determined by a new colorimetric method. *Clinica Chimica Acta, 90*, 37-43. http://dx.doi.org/10.1016/0009-8981(78)90081-5

Scalbert, A., Manach, C., Morand, C., Rémésy, C., & Jiménez, L. (2005). Dietary polyphenols and the prevention of diseases. *Critical Reviews in Food Science and Nutrition, 45*, 287-306. http://dx.doi.org/10.1080/1040869059096

Scott, G. (1997). *Antioxidants in Science, Technology, Medicine and Nutrition.* Albion Publishing, Chichester: England.

Singleton, V. L., &Rossi Jr, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture, 16*, 144-158.

Sinha, K. A. (1972). Colorimetric assay of catalase. *Analytical Biochemistry, 47*, 389-394. http://dx.doi.org/10.1016/0003-2697(72)90132-7

Tchiégang, C., & Mbougueng, P. D. (2005). Chemical composition of spices used in the cooking of Nah poh and Nkui of western cameroon. *Tropicultura, 23*, 193-200, in French.

Teissedre, P. L., & Landrault, N. (2000). Wine phenolics: contribution to dietary intake and bioavailibility. *Food Research International, 33*, 461-467. http://dx.doi.org/10.1016/S0963-9969(00)00070-3

Tulipani, S., Romandini, S., Busco, F., Bompadre, S., Mezzetti, B., & Battino, M. (2009). Ascorbate, not urate, modulates the plasma antioxidant capacity after strawberry intake. *Food Chemistry, 117*, 181-188. http://dx.doi.org/10.1016/j.foodchem.2009.03.096

Vanamala, J., Kester, A. C, Heuberger, A. L., & Reddivari, L. (2012). Mitigation of Obesity-Promoted Diseases by Nigella sativa and Thymoquinone. *Plant Foods for Human Nutrition*. http://dx.doi.org/10.1007/s11130-012-0279-z

World Health Organization. (1990). *Diet, nutrition and the prevention of chronic disease. Technical report series, 797*. Geneva: World Health Organization.

Yesilbursa, D., Serdar, Z., Serdar, A., Sarac, M., & Jale, C. (2005). Lipid peroxides in obese patients and effects of weight loss with orlistat on lipid peroxides levels. *International Journal of Obesity, 29*, 142-145. http://dx.doi.org/10.1038/sj.ijo.0802794