Preventive Medicine 54 (2012) S90–S97

Contents lists available at SciVerse ScienceDirect

Preventive Medicine

journal homepage: www.elsevier.com/locate/ypmed

Effects of a short term supplementation of a fermented papaya preparation on biomarkers of diabetes mellitus in a randomized Mauritian population

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ARTICLE INFO

Available online 11 February 2012

Keywords:
Neo-diabetics
Clinical trial
Fermented papaya preparation (FPP)
Antioxidants
Biomarkers

ABSTRACT

Objective. Clinical evidence and cellular models have shown an inverse relationship between the intakes of plant and fruit based diets and oxidative stress, suggesting the suitability of natural antioxidants in the management of diabetes mellitus and its complications.

Method. A randomized controlled clinical trial was conducted at the Cardiac Centre, SSRN Hospital, Pamplemousses, (Mauritius) to determine the effect of a short term supplementation of a fermented papaya preparation (FPP®) on biomarkers of diabetes and antioxidant status in a multi-ethnical neo-diabetic population from November 2010 to March 2011.

Result. Supplementation of 6 g FPP®/day for a period of 14 weeks could improve the general health status of several organs targeted by oxidative stress during diabetes. When comparing experimental to control groups with independent samples *t*-test, C-reactive protein levels significantly decreased ($P = 0.018$), LDL/HDL ratio was considerably changed ($P = 0.042$), and uric acid levels were significantly improved ($P = 0.001$). ANOVA results also validated the same findings with significant differences in C-reactive protein, LDL/HDL ratio, uric acid and in serum ferritin levels.

Conclusion. FPP® may present a novel, economically feasible nutraceutical supplement for the management of diabetes and for those at risk for cardiovascular disease, neurological disease and other conditions worsened by overt inflammation and oxidative stress.

Introduction

In order to realize the benefits of the application of nutraceuticals for the management of diabetes mellitus, cardiovascular dysfunction and neurodegenerative diseases, both scientists and consumers must go beyond the simplistic claims of positive properties. A clear understanding of the basic molecular mechanisms of dietary antioxidants that may prevent or reverse the progression of diseases balanced with clinical evidence of efficacy is of paramount great importance. The island of Mauritius has a high prevalence of diabetes amongst its population, with a toll of 22.6% and 21.2% of deaths related to diabetes and cardiovascular diseases respectively (Health Statistics Report, 2007). Indeed ethnic variation amongst inhabitants of specific geographic regions, gender, diet, and body mass index have all proven to be variables in the development of neurodegeneration, cardiovascular disease and the development of diabetes mellitus. Despite the discovery of natural and synthetic hypoglycemic agents, diabetes remains an issue of global concern that threatens to overwhelm national healthcare systems. Interest into the phenomenon of oxidative stress as a target of disease prevention has risen from the recognition that oxidative stress is a hallmark in the spread of degenerative\ diseases such as diabetes (Araki and Nishikowa, 2010), cancer (Visconti et al., 2009), Alzheimer's (Markesberry, 1997), chronic inflammation and cardiovascular disorders (Gleissner et al., 2007). Free radicals and oxidative stress are associated with morphological and metabolic derangements in major organs causing secondary complications to arise during diabetes (Gleissner et

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doi:10.1016/j.ypmed.2012.01.014

al., 2007; Aruoma and Halliwell, 1998). Fermented Papaya Preparation (FPP®) is a dietary product developed by Osato Research International, Japan, using a novel biofermentation technique. The functional benefits and composition of FPP® have been reviewed by Aruoma et al. (2010). In the present study the effects of a short term supplementation of FPP® on selected biomarkers of diabetes were assessed in a randomized neo-diabetic Mauritian population.

Methodology

Subject recruitment

For this study, 127 Mauritian neo-diabetic subjects, the majority of who actively participated in a nationwide survey organized by the Non-Communicable Diseases Unit of the Ministry of Health and Quality of Life in 2009, were recruited. The inclusion criteria were (1) fasting blood glucose range 5.1–5.9 mmol/L (2) age range 25–60 years with no secondary complications (3) non-smoker or stopped for more than 6 months (4) alcoholic consumption less than 2 standard drinks/day (5) post-menopausal women not receiving hormone replacement treatment (6) not receiving glucose-lowering, cholesterol-lowering or anti-hypertension treatment. Written consent was obtained from all subjects prior to the study, which was conducted in accordance to guidelines set by the National Ethics Committee of the Ministry of Health and Quality of Life (Republic of Mauritius).

Study design

The study consisted of a randomized, controlled clinical trial (clinicaltrial.gov identifier NCT01248143) with treatment and control groups running in parallel for a period of 16 weeks. A simple randomization approach with no blocking was used to allocate subjects. The treatment group consumed 1 standard sachet of FPP® dissolved in half a glass of warm water twice daily before meals for 14 weeks followed by a 2 week wash out period of consuming the same amount of water/day. The control group consumed an equivalent amount of water. Subjects were asked to maintain their usual diet and physical activity while enrolled and keep a detailed record of all food and beverage consumed during main mealtimes (breakfast, lunch and dinner) over the study period.

Blood collection and analysis

Blood and urine samples (20 mL) were collected at the Cardiac Centre, SSRN hospital, at baseline, week 14 and after wash out following a 10 hour fast by the subjects. Samples were analyzed using a fully automated clinical chemistry analyzer (Olympus AU480, Beckman Coulter® Inc.) at the Apollo Bramwell Hospital, Mauritius. Serum or EDTA-plasma were tested for glucose, total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, C-reactive protein (CRP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), ferritin, uric acid, total antioxidant status (TAS; NX2332) and glycated hemoglobin (HbA1c). Microalbumin and urinary creatinine levels were also examined. Commercial reagent kits were purchased from Beckman Coulter® and Randox® laboratories. At each blood collection, 25 blood and urine samples were randomly selected and sent to an independent laboratory (Biohealth Ltd., Mauritius) for cross-checking.

Survey of diet forms

All subjects were given a dietary questionnaire on which all food and beverage consumed during the study was recorded. Statistical analysis of the diet forms was based on the lipid/fat ratio of each item (USDA, 2010). The mean diet score value/week was recorded and the trend observed throughout the intervention period.

Statistical analysis

Data is expressed as mean ± standard deviation. Both descriptive and inferential statistical analyses were carried out using Microsoft Excel and MedCalc® (version 11.5.1). Shapiro–Wilk's test was used to define the normality of data. Where data was normal, tests of significance of observed mean differences over the intervention period for 2 sets of data was performed using the Student's paired *t*-test. Its non-parametric alternate, the Wilcoxon test, was used for non-normal data. Data was entered into SPSS (version 14.0) for further analysis. Differences were considered to be significant when two-tailed $P<0.05$.

Results

The number of subjects recorded at the end of the study was 101, representing a 20% drop out from the initial population. Reasons for drop out were mainly occupational constraints. No adverse effects were reported during the intervention period.

Diet score

The diet score calculated reflected the lipid/fat content of food consumed on a weekly basis during the study. Baseline scores varied from 108 to 289 arbitrary units in males and 122 to 350 arbitrary units in females of the treatment group. Fig. 1 shows that there were minor variations of mean scores from baseline to wash out.

Physical parameters

Systolic blood pressure was observed to decrease significantly ($P<0.05$) in males of the FPP® study group by 4.4% compared to the controls. This change was evident only after wash out treatment. In females, both systolic and diastolic blood pressure dropped by 1.8% ($P>0.05$) and 2% ($P<0.05$) respectively at week 14 of the study. FPP® supplementation had no apparent effect on other physical parameters (Table 1).

Biomarker analysis

Fasting blood glucose and glycated hemoglobin (HbA1c)

Mean fasting blood glucose levels of the treatment group at baseline ranged from 73 to 151 and 84 to 111 mg/dL in males and females respectively where levels greater than 126 mg/dL are considered pathological. These values remained relatively unchanged in both genders of treatment and control groups (Table 2). FPP® had no influence on glycated hemoglobin levels but slight non-significant changes could be noted throughout supplementation, this trend being also observed in control group.

Lipid profile

Total cholesterol (TC) levels ranged from 128 to 298 and 142 to 280 mg/dL in males and females respectively at baseline. A drop of 4.2% and 3.5% in males and females was observed at week 14, which was not statistically significant (Table 3). Wash out treatment caused total cholesterol level to drop further by 4.4% in males. HDL cholesterol and LDL/HDL cholesterol ratios remained unchanged by FPP® consumption, whereas LDL cholesterol levels generally decreased in the treatment group. Basal serum triglyceride levels ranged from 22 to 288 mg/dL in the sample population, where levels below 150 mg/dL are targeted. Short term FPP® intake apparently caused triglyceride levels to rise by 30.5% ($P<0.05$) and 7% ($P<0.05$) in males and females respectively at week 14. After wash out, triglycerides dropped non-significantly. A *t*-test for independent samples indicated an overall statistically significant difference between pre and post supplementation

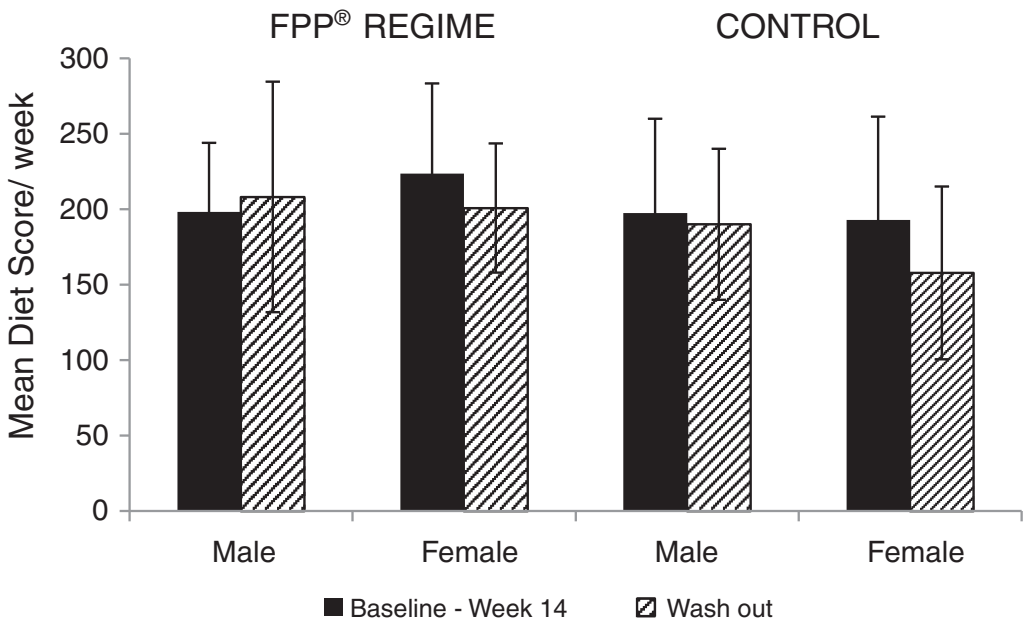

Fig. 1. Diet scores of a neo-diabetic male and female Mauritian population during the intervention period from November 2010 to March 2011.

groups when compared to control at (P = 0.04) (Table 5) and using ANOVA between and within groups (P = 0.04) (Table 6).

Uric acid

Baseline concentrations ranged between 4.1 and 6.1 mg/dL and 0.8 and 8.2 mg/dL in males and females of the treatment group. Concentrations greater than 7.2 mg/dL (males) and 6.0 mg/dL (females) predict a high risk of developing diabetes, hypertension and/or cardiovascular dysfunction. Serum uric acid levels remained relatively unchanged in both the FPP® and control groups after wash out (Table 4). No statistically significant changes could be noted (Table 4). When comparing uric acid levels pre and post-supplementation between experimental and control groups, the difference was statistically significant (P = 0.001) (Table 5). An ANOVA validated the significant differences between groups (P = 0.001) (Table 6).

C-reactive protein (CRP)

CRP, an acute phase reactant of the liver, can be referred to as a marker of inflammation in diabetes, cardiovascular disease and in the elderly for levels greater than 5 mg/L. In this study baseline analysis showed CRP concentrations to range from 0.4 to 9.5 and 0.4 to 23.4 mg/L in males and females respectively. Comparing experimental and control groups for pre and post-supplementation results, the change in CRP was found to have significantly dropped in the experimental group (P = 0.018) (Table 5). An ANOVA further validates significance between groups (P = 0.001) (Table 6). FPP® consumption caused CRP to drop but the findings over this period of time was not statistically significant varying in males by 13.3% at week 14 however after washout, levels rose significantly by 45.6% (P<0.05). Although CRP levels remained constant in females, it was statistically not significant (Table 4), suggesting further studies that needs to include population with defined pathology and controls.

Table 1
Effect of a fermented papaya preparation on various physical parameters in a neo-diabetic male and female Mauritian population under the FPP® and control regimens during the intervention period from November 2010 to March 2011 (*P<0.05, **P<0.01, ***P<0.001).

Physical parameters	Gender	N	FPP® regime			N	Control		
			Mean ± SD baseline value	Mean ± SD week 14 value	Mean ± SD wash-out value		Mean ± SD baseline value	Mean ± SD week 14 value	Mean ± SD wash-out value
BMI (Body Mass Index)	Male	24	26.753 ± 2.638	26.891 ± 2.566 (+0.5%)	26.915 ± 2.566 (+0.1%)	29	26.306 ± 4.765	26.517 ± 4.791 (+0.8%)	26.387 ± 4.793 (−0.5%)
	Female	20	26.895 ± 3.503	27.29 ± 3.746 * (+1.5%)	27.157 ± 3.671 (−0.5%)	27	26.547 ± 3.346	26.483 ± 3.217 (−0.2%)	26.593 ± 3.306 (+0.4%)
Waist/Hip ratio	Male	24	0.92 ± 0.052	0.911 ± 0.048 (−1%)	0.916 ± 0.057 (+0.5%)	29	0.918 ± 0.055	0.909 ± 0.058 (−1%)	0.902 ± 0.045 (−0.8%)
	Female	20	0.823 ± 0.047	0.84 ± 0.074 (+2.1%)	0.841 ± 0.062 (+0.1%)	27	0.825 ± 0.047	0.839 ± 0.059 (+1.7%)	0.846 ± 0.0319 (+0.8%)
Systolic blood pressure	Male	22	131.682 ± 11.032	131.455 ± 11.393 (−0.2%)	125.674 ± 9.515 * (−4.4%)	28	128.357 ± 11.831	124.679 ± 11.257 * (−2.9%)	123.339 ± 11.760 (−1.1%)
	Female	19	127.068 ± 16.071	124.789 ± 19.997 (−1.8%)	123.605 ± 12.449 (−0.9%)	26	120.038 ± 9.256	117.077 ± 9.469 (−2.5%)	118.348 ± 9.256 (+1.1%)
Diastolic blood pressure	Male	24	82.5 ± 7.476	80.104 ± 7.476 (−2.9%)	78.767 ± 5.874 (−1.7%)	28	80.5 ± 7.285	76.621 ± 8.559 * (−4.8%)	76.668 ± 7.856 (+0.1%)
	Female	19	75.063 ± 9.366	73.55 ± 13.012 * (−2%)	73.079 ± 8.878 (−0.6%)	26	74.591 ± 6.150	71.058 ± 7.895 (−4.7%)	71.654 ± 7.107 (+0.8%)

FPP®: fermented papaya preparation; *N*: number of subjects; SD: standard deviation

Table 2
Effect of a fermented papaya preparation on fasting blood glucose levels and percentage of glycated hemoglobin in a neo-diabetic male and female Mauritian population under the FPP® and control regimens during the intervention period from November 2010 to March 2011 (*P<0.05, **P<0.01, ***P<0.001).

Biomarkers	Gender	N	FPP® regime			N	Control		
			Mean ± SD baseline value	Mean ± SD week 14 value	Mean ± SD wash-out value		Mean ± SD week 14 value	Mean ± SD wash-out value	Mean ± SD baseline value
Fasting glucose (mg/dL)	Male	21	94.667 ± 8.218	96.25 ± 16.999 (+1.7%)	94.818 ± 9.903 (−1.5%)	25	90.84 ± 7.658	87.556 ± 11.659 (−3.6%)	90.519 ± 9.924 (+3.4%)
	Female	19	97.1 ± 6.656	95.65 ± 15.832 (−1.5%)	91.263 ± 12.670 (−4.6%)	26	91.5 ± 9.705	94.286 ± 5.041 (+3.0%)	91.038 ± 8.811** (−3.4%)
Glycated hemoglobin (%)	Male	23	6.03 ± 0.526	6.017 ± 0.613 (−0.2%)	6.1 ± 0.619 ** (+1.4%)	27	5.952 ± 0.378	5.868 ± 0.335	5.893 ± 0.317
	Female	19	6.042 ± 0.445	6.145 ± 0.409 (+1.7%)	6.23 ± 0.485 (+0.4%)	25	5.928 ± 0.235	5.944 ± 0.245 (+0.3%)	6.031 ± 0.262 *** (+1.5%)

FPP®: fermented papaya preparation; *N*: number of subjects; SD: standard deviation

Ferritin

Studies investigating iron overload in the pancreas as a risk factor for diabetes has gained renewed interest. Normal ferritin ranges are 3–552 ng/mL in adult males and 6–159 ng/mL for women. In this study, baseline concentrations were 3–289 ng/mL and 2–144 ng/mL in males and females respectively. Ferritin levels between groups were found to have statistically significant differences (P = 0.012) (Table 6).

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST)

The liver is an important site of glucose level maintenance and insulin clearance. Declining liver functionality during diabetes can be associated to elevated ALT, and to a lesser extent AST. In this study, the possible protective effect of FPP® on the liver was more evident in females of both treatment and control groups. In this study, the possible effect of FPP® consumption on ALT and AST levels was more pronounced in females.

Levels dropped non-significantly to 14.444 ± 3.585 IU/L (−12.6%) and 19.056 ± 4.108 IU/L (−8.8%) respectively compared to baseline. Both ALT and AST concentrations were observed to slightly increase in both males and females (P<0.05) of the treatment group after wash out (Table 4).

Microalbumin to urinary creatinine ratio

Microalbumin/urinary creatinine ratio was used to assess the effect of FPP® on the improvement of kidney functionality in pre-diabetic subjects. Basal ratios ranged from 2.8 to 22.1 in males and 2.4 to 13.5 in females of the treatment group. At week 14, ratio decreased by 21.8% and 28.6% in males of the treatment and control group respectively. Further non-significant decreases were noted after wash out treatment. Ratio changes in females were pronounced only after wash out period (Table 4).

Total antioxidant status

Mean plasma basal TAS values ranged from 1.31 to 2.11 mmol/L. Post wash out analysis showed supplementation with 6 g FPP®/day markedly and significantly increased antioxidant status in both genders of the treatment and control group (P<0.001, Fig. 2).

Discussion

With the rising cost of health care and pharmacological interventions, the domain of functional foods in the prevention of diabetes and cardiovascular diseases has gained special attention from both the scientific community and consumers. Functional foods are amendable to clinical trial, but the challenge of translating scientific

Table 3
Effect of a fermented papaya preparation on the lipid profile in a neo-diabetic male and female Mauritian population under the FPP® and control regimens during the intervention period from November 2010 to March 2011 (*P<0.05, **P<0.01, ***P<0.001).

Biomarkers	Gender	N	FPP® regime			N	Control		
			Mean ± SD baseline value	Mean ± SD week 14 value	Mean ± SD wash-out value		Mean ± SD week 14 value	Mean ± SD wash-out value	Mean ± SD baseline value
Total cholesterol (mg/dL)	Male	24	209.333 ± 42.809	200.5 ± 43.064 (−4.2%)	191.625 ± 43.725 (−4.4%)	28	215.393 ± 34.133	184.862 ± 35.338* (−12.6%)	194.103 ± 42.058 (+5%)
	Female	20	204.45 ± 36.543	197.85 ± 37.550 (−3.5%)	200.85 ± 30.114 (+1.8%)	26	205.321 ± 47.519	181.231 ± 32.817*** (−11.7%)	194.036 ± 32.343 * (+7.1%)
HDL cholesterol (mg/dL)	Male	23	40.391 ± 6.713	40.458 ± 7.599 (+0.2%)	40.417 ± 7.359 (−0.1%)	28	45.536 ± 9.414	39.241 ± 8.361*** (−13.8%)	41.103 ± 9.194 (+4.7%)
	Female	19	50 ± 8.510	45.75 ± 8.441 (−8.5%)	47.053 ± 5.400 (+2.8%)	24	50.208 ± 10.129	48.885 ± 10.603 ** (−6.6%)	46.16 ± 9.186 (−1.5%)
LDL cholesterol (mg/dL)	Male	22	144.167 ± 32.921	134.75 ± 33.604 (−6.5%)	132 ± 38.659 (−2%)	28	143.643 ± 30.894	126.071 ± 28.887* (−12.2%)	135.25 ± 35.874* (+7.3)
	Female	17	135.263 ± 28.051	128.118 ± 19.551 (−5.3%)	139.316 ± 20.379 * (+8.7%)	25	135.222 ± 38.551	117.2 ± 27.735 *** (−13.3%)	128.815 ± 26.205 *** (+9.9%)
LDL/HDL ratio	Male	23	3.477 ± 0.208	3.387 ± 0.849 (−2.6%)	3.341 ± 0.949 (−1.4%)	29	3.3 ± 1.131	3.241 ± 0.391 (−1.8%)	3.368 ± 0.541 (+3.9%)
	Female	19	2.923 ± 0.906	2.829 ± 0.663 (−3.2%)	2.917 ± 0.509 (+3.1%)	27	2.629 ± 0.626	2.507 ± 0.382 (−4.6%)	2.629 ± 0.389 (+4.87%)
Triglyceride (mg/dL)	Male	22	147.136 ± 61.614	192.083 ± 91.676 * (+30.5%)	164.435 ± 68.226 (−14.4%)	25	130.44 ± 54.776	124 ± 52.626 (−4.9%)	103.88 ± 33.175* (−16.2%)
	Female	19	121.95 ± 64.389	130.526 ± 56.451 (+7%)	125.158 ± 62.708 (−4.1%)	24	93.680 ± 33.532	102.08 ± 35.402 (+9%)	97.667 ± 37.024 (−4.3%)

FPP®: fermented papaya preparation; *N*: number of subjects; SD: standard deviation; HDL: high-density lipoprotein; LDL: low-density lipoprotein

Table 4
Effect of a fermented papaya preparation on the levels of AST, ALT, ferritin, CRP, uric acid, microalbumin/urinary creatinine ratio in a neo-diabetic male and female Mauritian population under the FPP® and control regimens during the intervention period November 2010 to March 2011 (*P<0.05, **P<0.01, ***P<0.001).

Biomarkers	Gender	N	FPP® regime			N	Control		
			Mean ± SD baseline value	Mean ± SD week 14 value	Mean ± SD wash-out value		Mean ± SD baseline value	Mean ± SD week 14 value	Mean ± SD wash-out value
AST (IU/L)	Male	22	24.182 ± 4.563	25.091 ± 5.756 (+3.6%)	26.045 ± 5.948 (+4.2%)	27	26.333 ± 6.239	26.071 ± 76.242 (−1%)	27.107 ± 7.208 (+4%)
	Female	18	20.895 ± 5.425	19.056 ± 4.108 (−12.6%)	20.053 ± 5.671 (−2.9%)	25	22.143 ± 5.602	21.385 ± 4.196 (−3.4%)	21.64 ± 3.946 (+1.2%)
ALT (IU/L)	Male	22	24.091 ± 8.518	22.955 ± 8.459 (+3.6%)	26 ± 8.639 (+4.2%)	28	29.138 ± 13.624	27.536 ± 11.325 (−5.5%)	28.607 ± 12.249 (+3.9%)
	Female	17	16.526 ± 5.611	14.444 ± 3.585 (−12.6%)	15.824 ± 5.175 * (+9.6%)	25	18.464 ± 7.796	16.192 ± 4.674 (−12.3%)	15.72 ± 4.642 (−2.9%)
Ferritin (ng/ml)	Male	19	102.263 ± 75.981	121.957 ± 71.886 (+19.3%)	135.417 ± 81.075 (+11%)	25	80.038 ± 47.118	106.577 ± 61.397 (+33.2%)	98.88 ± 58.648 (−7.2%)
	Female	18	32.611 ± 36.914	47.053 ± 49.396 (+44.3%)	45.789 ± 50.879 (−2.7%)	26	42.731 ± 42.053	40.038 ± 35.603 (−6.3%)	45.385 ± 41.968 * (+13.4%)
C-reactive protein (mg/L)	Male	21	1.519 ± 1.115	1.317 ± 0.715 (−13.3%)	1.918 ± 1.471* (+45.6%)	23	1.327 ± 0.877	1.174 ± 0.466 (−11.5%)	0.983 ± 0.306* (−16.4%)
	Female	19	3.168 ± 6.407	4.921 ± 3.659* (+55.3%)	4.216 ± 3.298 (−14.3%)	24	2.963 ± 2.669	2.692 ± 2.663 (−9.1%)	2.304 ± 1.876 (−14.4%)
Uric acid (mg/dL)	Male	24	6.654 ± 1.472	6.525 ± 1.315 (−1.9%)	6.604 ± 1.238 (+1.2%)	26	5.90 ± 1.519	5.793 ± 0.107 (−1.8%)	5.635 ± 0.852 (−2.7%)
	Female	18	4.55 ± 0.901	4.884 ± 0.700 (+7.3%)	4.835 ± 0.770 (−1%)	27	4.569 ± 0.742	4.529 ± 0.731 (−0.9%)	4.596 ± 0.722 (+2.8%)
Microalbumin/urinary creatinine ratio	Male	20	6.24 ± 4.092	4.88 ± 4.299 (−21.8%)	3.165 ± 1.468* (−35.1%)	25	5.307 ± 3.844	3.788 ± 1.444* (−28.6%)	3.392 ± 1.324 (−10.5%)
	Female	16	5.856 ± 5.982	10.779 ± 8.391 (+84.1%)	4.794 ± 2.191* (−55.5%)	23	7.204 ± 4.948	6.629 ± 4.484 (−8%)	7.478 ± 5.728 (+12.8%)

FPP®: fermented papaya preparation; *N*: number of subjects; SD: standard deviation; AST: aspartate aminotransferase; ALT: alanine aminotransferase

evidence into economically feasible solutions to mitigate the current health situation remains questionable. Metabolic syndrome, a major health problem in developed countries including Mauritius, is a constellation of risk factors that include low HDL, high triglyceride, impaired fasting glucose and central adiposity which predispose to the high incidence of type 2 diabetes, atherosclerosis and other oxidative stress related diseases (Chen et al., 2008; The trends in diabetes and cardiovascular diseases risk in Mauritius, 2009). This is the first study investigating the bioefficacy of the dietary supplement FPP® on a wide range of biomarkers related to diabetes in a random population of neo-diabetic individuals. Elevated blood pressure is commonly experienced during diabetes; it is likely that the interaction of excessive superoxide radicals with endothelial nitric oxide can influence vascular smooth muscle contraction (Cuzzocrea et al., 2004). As a general decline in blood pressure was observed after FPP® consumption our study suggests that FPP® can provide vascular protection in cases of elevated blood pressures. It is highly probable that this protective effect may be due to its polyphenols, as was reported by Aviram et al. (2004) in which pomegranate juice, a rich source of tannins and anthocyanidins, lowered systolic blood pressure by 12% (P<0.05) in a cohort of atherosclerotic patients. A recent meta-analysis also revealed a strong correlation between the consumption of coca-rich products (e.g. black chocolate) and lowered blood pressure, a trend suspected to be influenced by its high flavonoid content (Desch et al., 2010).

Table 5
Effect of a fermented papaya preparation on a neo-diabetic Mauritian population under the FPP® and control regimens (*N* = 41) during the intervention period November 2010 to March 2011 Significance testing as Independent samples *T*-test amongst the pre and post supplementation groups when comparing experimental and control groups (*P<0.05, **P<0.01. ***P<0.001).

Biomarker	FPP® regime		Control regime	
	F-value	Significance	F-value	Significance
LDL/HDL ratio	4.393	0.042*	0.285	0.596
CRP	6.078	0.018*	7.414	0.190
Uric acid	12.550	0.001***	0.910	0.345

FPP®: fermented papaya preparation; *N*: number of subjects;LDL/HDL: ratio of low-density lipoprotein to high-density lipoprotein; CRP: C-reactive protein

Fasting blood glucose was slightly decreased with FPP® consumption in which the differences were found to be not statistically significant (Table 2). The trend observed is similar to the previous clinical trial conducted by Danese et al. (2006) in which both fasting and post-meal glucose dropped significantly (P<0.001) after intake of 3 g FPP®/day for 2 months in a cohort of type 2 diabetic patients, claiming FPP® to work in synergy with oral hypoglycemic drugs. The hypoglycemic effect of FPP® was recently confirmed in diabetic db/db mice supplemented 0.2 g/kg body weight for 8 weeks (Collard and Roy, 2010).

A growing database of clinical evidence suggests that abdominal obesity is a stronger predictor of diabetes than BMI and waist/hip ratio. Results of this study and that of Collard and Roy (2010) showed that short term FPP® intake does not cause weight gain in diabetic subjects. An improvement in the overall lipid profile after FPP® intake could be noted. Interestingly significant changes were also observed in the control group, notably the total cholesterol LDL cholesterol and the triglyceride levels (Table 3) questioning the inertness of hot water.

Uric acid has an undefined role in the body. It may cause deleterious effects on the endothelium through oxidative stress, major steps in the progression of atherosclerosis. Since our baseline uric acid levels were relatively high in males (Table 4), the likelihood of these neo-diabetic males of developing vascular disease is quite strong. Elevated uric acid, urea and urinary albumin and creatinine levels during insulin resistance in overweight and hypertensive subjects may be of major concern. Santiago et al. (1994) have shown that a short term intake of FPP® could improve renal integrity, thus the possible correlations with inflammatory cytokines deserves further investigation.

C-reactive protein, an acute phase reactant of the liver, is routinely used to detect inflammation and to some extent positively correlated to oxidative stress. Sattar et al. (2004) reported a possible correlation with tumor necrosis factor-α (TNF-α) and BMI. In this study, baseline CRP levels and BMI in females were much higher compared to males

Table 6
ANOVA testing on the effect of a fermented papaya preparation on a neo-diabetic Mauritian population under the FPP® and control regimens during the intervention period from November 2010 to March 2011 ($N = 48$; *P<0.05, **P<0.01. ***P<0.001).

Biomarkers		Sum of squares	df	Mean square	F	Sig.
Glucose	Between groups	67.756	2	33.878	.085	.919
	Within groups	26028.008	65	400.431		
	Total	26095.765	67			
Total cholesterol	Between groups	1169.808	2	584.904	.373	.690
	Within groups	102033.883	65	1569.752		
	Total	103203.691	67			
HDL	Between groups	223.459	2	111.729	1.349	.267
	Within groups	5383.286	65	82.820		
	Total	5606.745	67			
LDL	Between groups	1183.496	2	591.748	.628	.537
	Within groups	61282.783	65	942.812		
	Total	62466.279	67			
LDL/HDL ratio	Between groups	6.870	2	3.435	3.405	*.039
	Within groups	65.575	65	1.009		
	Total	72.445	67			
Triglycerides	Between groups	28206.988	2	14103.494	1.572	.216
	Within groups	583308.542	65	8973.978		
	Total	611515.529	67			
AST	Between groups	7421.418	2	3710.709	.967	.386
	Within groups	249351.700	65	3836.180		
	Total	256773.118	67			
ALT	Between groups	15984.798	2	7992.399	.989	.377
	Within groups	525092.967	65	8078.353		
	Total	541077.765	67			
CRP	Between groups	175.693	2	87.847	7.426	***.001
	Within groups	768.956	65	11.830		
	Total	944.649	67			
Microalbumin	Between groups	265.849	2	132.925	.263	.770
	Within groups	32868.783	65	505.674		
	Total	33134.632	67			
Urinary creatinine	Between groups	50110.231	2	25055.116	4.000	*.023
	Within groups	407112.533	65	6263.270		
	Total	457222.765	67			
Ferritin	Between groups	81924.503	2	40962.252	4.256	*.018
	Within groups	606392.164	63	9625.272		
	Total	688316.667	65			
Uric acid	Between groups	43.662	2	21.831	14.068	***.000
	Within groups	100.870	65	1.552		
	Total	144.532	67			
TAS	Between groups	.053	1	.053	1.879	.178
	Within groups	1.137	40	.028		
	Total	1.191	41			
HbA1C	Between groups	.184	1	.184	.586	.448
	Within groups	13.222	42	.315		
	Total	13.406	43			

FPP®: fermented papaya preparation; *N*: number of subjects; SD: standard deviation; df: degree of freedom; HDL: high-density lipoprotein; LDL: low-density lipoprotein; AST: aspartate aminotransferase; ALT: alanine aminotransferase; CRP: C-reactive protein; TAS: total antioxidant status; HBA1C: glycated hemoglobin.

(Table 4). Belalcazar et al. (2010) have reported that lifestyle intervention for weight loss in Individuals with type 2 diabetes reduces high CRP and suggests CRP as a potential predictor of metabolic changes. Further, the review paper of Danesh and Kanwar (2004) discussed the work of Pasceri et al. (2001) which pointed out the potential of statin drugs modulating the CRP induced inflammation thus leading us to suggest a potential adjunct benefit of FPP® that may augment the chemotherapeutic outcomes of statin therapy.

Accumulation of iron in the heart, liver and endocrine glands results in severe damage and eventual organ failure. Excess iron deposition in the pancreas, assessed by serum ferritin, is strongly associated to newly diagnosed diabetes in both males and females (Ford and Cogswell, 1999). In the present study, baseline ferritin levels were relatively high in both groups. High iron body stores or a delay in glycosylated ferritin clearance may be possible explanations (Ford and Cogswell, 1999). The intake of FPP® is widely reported to contribute to the alleviation of oxidative stress-associated symptoms in blood disorders. (Amer et al., 2008; Ghoti et al., 2010; Rund and Rachmilewitz, 2005).

An average 34.9% of the Mauritian adult population between 25 and 75 years is overweight (The trends in diabetes and cardiovascular diseases risk in Mauritius, 2009). This raises concern given the numerous cases of diabetes and non-alcoholic fatty liver disease (NALFD) which prevails within this age range (Marchesini et al., 2001). Elevated enzymes such as γ-glutamyltransferase, ALT and to a lesser extent AST can provide an insight into the pathology of the liver, thus predicting risk of developing NALFD and/or type 2 diabetes (Sattar et al., 2004; Vozarova et al., 2002). This study showed that FPP® consumption could greatly reduce circulating ALT and AST levels, thus improve hepatic insulin sensitivity in high risk groups. This trend was also reported by Santiago et al. (1994) in 3 unhealthy adults supplemented with FPP® for a period of 14 days. The simplicity of ALT and AST measurement suggests its inclusion during routine screening.

Total antioxidant statuses markedly and significantly increased throughout the intervention period. A high plasma antioxidant level offers maximum protection against oxidative stress through quenching of hydroxyl radicals and nitric oxide (Zhang et al., 2006). FPP® and *Manda* (a fermented mixture of fruits, marine algae, cereal grains and marine algae) could equally provide a barrier against lipid peroxidation in senescent rat brains, thus suppressing epileptic seizures (Kawai et al., 1998; Santiago et al., 1992). Activation of the hydroxyl, thiol and

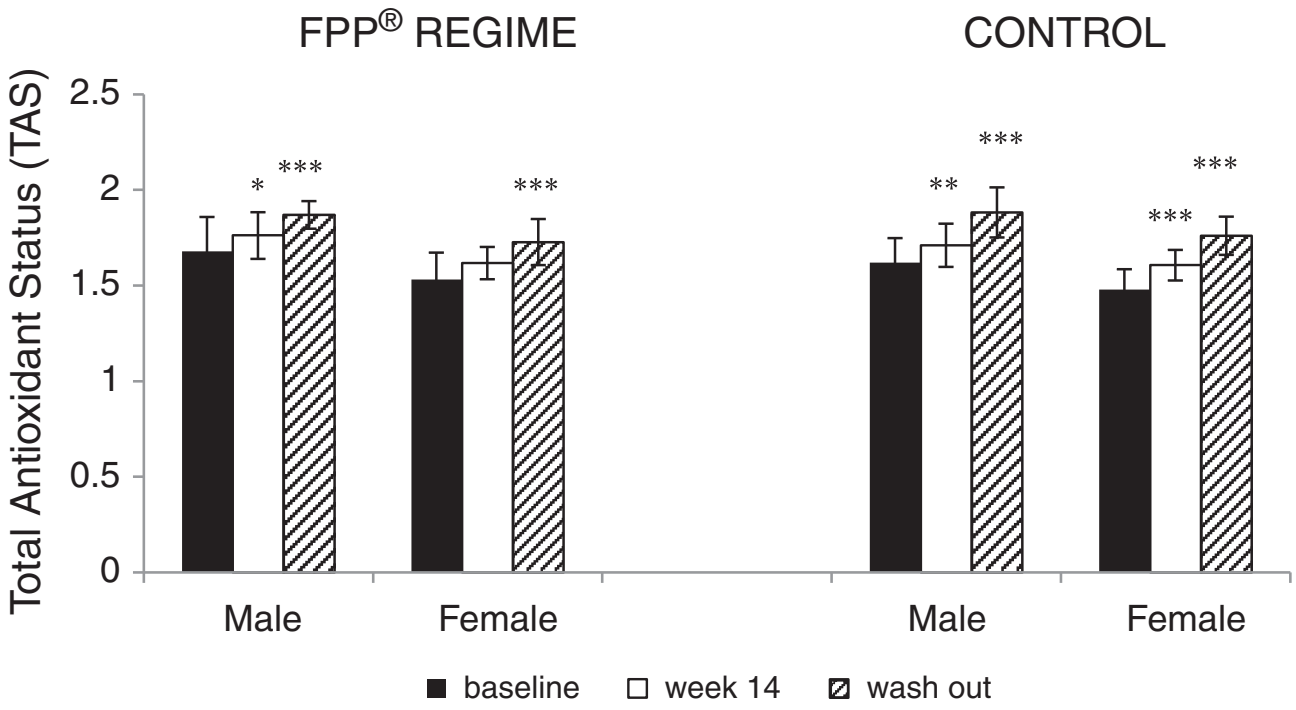

Fig. 2. Effect of a fermented papaya preparation on the total antioxidant status in a neo-diabetic male and female Mauritian population under the FPP® and control regimens during the intervention period from November 2010 to March 2011(*P<0.05, **P<0.01, ***P<0.001).

sulfhydryl groups of the several amino acids present in FPP® are believed to contribute to its overall antioxidant potency in the organ tissues after uptake (Osato et al., 1995).

Although several strengths of this study were namely, the participation of a multi-ethnical population with varied dietary regime (vegetarians and non-vegetarians) of average age 48 (In Mauritius about 1 in every 3 adults above the 35) has diabetes (The trends in diabetes and cardiovascular diseases risk in Mauritius, 2009) and the fact that drug interaction was not a confounding factor. Limitations were the short study duration of 14 weeks and the dosage. Higher doses of FPP®, a larger sample population and a longer intervention period may be required to show significant clinical improvements. The information gained from this study should be used to comprehensively build a portfolio of markers that should be integrated into routine clinical testing for diabetes and cardiovascular in at-risk individuals. This may then have a global application.

Conclusion

A 3 month supplementation of the fermented papaya preparation can generally improve the well-being of certain organs targeted by oxidative stress experienced during diabetes mellitus type 2 as indicated through the broad spectrum of biomarkers. However, considering the complexity of the mechanisms involved in the human body burdened by oxidative stress, and the lack of supplementary studies on our study population-it cannot be entirely concluded that 6 g FPP®/day for 3 months positively influences the inflammatory response and immune functioning as other studies revealed. Further, the view that statin drugs modulates CRP induced inflammation, leads us to suggest a potential adjunct benefit of FPP® that may augment the chemotherapeutic outcomes of statin therapy.

Conflict of interest statement

The authors JS, TKG, SK, VD, FM, KG, DD, JI, EB and TB declare no conflicts of interest. OIA is actively involved in biomedical research involving fermented papaya preparation.

Acknowledgments

This research was supported by the Mauritius Research Council and Osato Research Institute, Gifu, Japan. The authors would like to thank the staff of the Cardiac Centre, SSRN Hospital (Pamplemousses) and the technicians of the biochemistry laboratory of Apollo Bramwell Hospital and BioHealth Ltd (Beau Bassin).

This study is registered at www.clinicaltrial.gov (NCT01248143).

Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.ypmed.2012.01.014.

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