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Effects of *Hibiscus sabdariffa* extract powder and preventive treatment (diet) on the lipid profiles of patients with metabolic syndrome (MeSy)

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ABSTRACT

Insulin resistance, obesity, hypertension, and dyslipidemia are strongly associated with metabolic syndrome (MeSy), which is considered to be a reversible clinical stage before its evolution to coronary heart disease and diabetes. Currently, the antihypertensive and hypolipidemic properties of aqueous *Hibiscus sabdariffa* extracts (HSE) have been demonstrated in clinical trials and *in vivo* experiments. The aim of the present study was to evaluate the effects of a *Hibiscus sabdariffa* extract powder (HSEP) and a recognized preventive treatment (diet) on the lipid profiles of individuals with and without MeSy according to the National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATP III) criteria. The protocol was a follow-up study carried out in a factorial, randomized design (T1 = preventive treatment comprises Diet, T2 = HSEP, T3 = HSEP + preventive treatment (Diet) X MeSy, non-MeSy individuals). A total daily dose of 100 mg HSEP was orally administered in capsules for one month. The preventive treatment (diet) was selected according to NCEP-ATP III recommendations and adjusted individually. Total cholesterol, LDL-c, HDL-c, VLDL-c, triglycerides, glucose, urea, creatinine, AST, and ALT levels in the blood were determined in all individuals pre- and post-treatment. The MeSy patients treated with HSEP had significantly reduced glucose and total cholesterol levels, increased HDL-c levels, and an improved TAG/HDL-c ratio, a marker of insulin resistance (t-test $p < 0.05$). Additionally, a triglyceride-lowering effect was observed in MeSy patients treated with HSEP plus diet, and in individuals without MeSy treated with HSEP. Significant differences in total cholesterol, HDL-c, and the TAG/HDL-c ratio were found when the means of absolute differences among treatments were compared (ANOVA $p < 0.02$). Therefore, in addition to the well documented hypotensive effects of *Hibiscus sabdariffa*, we suggest the use of HSEP in individuals with dyslipidemia associated with MeSy.

Introduction

Metabolic syndrome (MeSy) has been identified as a cluster of several metabolic risk factors, including hypertension, insulin resistance (IR), dyslipidemia, excess adipose tissue, and markers of cardiovascular disease. In the Mexican population, a high frequency of MeSy has been reported compared to other populations (Aguilar-Salinas et al. 2004; Lorenzo et al. 2007; Ramírez-Vargas et al. 2007). The clinical importance of the detection and treatment of MeSy is clearly justified by the high risk of developing adverse outcomes, like cardiovascular disease and diabetes, in MeSy patients. Thus, some efforts are targeted to treat MeSy alterations prior to the evolution of chronic diseases. According to National Cholesterol Education Program (NCEP) Adult Treatment Panel (ATP)-III recommendations for MeSy management, these patients should initially be treated with more intensified therapeutic lifestyle changes, including diet and physical activity, rather than pharmacological therapy. Among

Abbreviations: MeSy, metabolic syndrome; HSEP, *Hibiscus sabdariffa* extract powder; HSE, *Hibiscus sabdariffa* extract; IR, insulin resistance; NCEP ATP-III, National Cholesterol Education Program Adult Treatment Panel III; BP, blood pressure; TC, total cholesterol; HDL-c, high-density lipoprotein-cholesterol; LDL-c, low-density lipoprotein-cholesterol; TAG, triglycerides; VLDL-c, very low-density lipoprotein-cholesterol; TAG/HDL-c, triglycerides/high density lipoprotein cholesterol ratio

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several treatments, herbal medicine represents a potentially effective approach because of its collateral effect reduction, commercial availability, and widespread use and high acceptance in folk medicine by the general public. In Mexico, *Hibiscus sabdariffa* L. (Mexican popular name: Jamaica) is consumed traditionally as a cold drink. The medical use of *Hibiscus sabdariffa* extracts (HSE) has been described in several studies, including as a diuretic, laxative, antibacterial agent, antioxidant agent, anti-hypertensive, and hypocholesterolemic, among others (Akindahunsi and Olaleye 2003; Herrera-Arellano et al. 2004, 2007; Hirunpanich et al. 2005, 2006). Recently, many investigations have used *in vivo* models, as well as clinical trials, for testing and characterizing the medical effects of HSE. A study with cholesterol-fed rabbits revealed the anti-atherosclerotic activity of HSE (Chen et al. 2003). The hypolipidemic and antioxidant effects of HSE were shown in an *in vivo* diabetic model (Farombi and Ige 2007). It has been demonstrated that anthocyanins, one of the major components of aqueous HSE, are responsible for the anti-hypertensive, antioxidant, and hypocholesterolemic effects of *Hibiscus Sabdariffa* (Chang et al. 2006; Herrera-Arellano et al. 2004; Hirunpanich et al. 2006). Taking together this evidence of the hypolipidemic efficacy of HSE, we hypothesized that treatment with *Hibiscus sabdariffa* could complement treatment in MeSy. On the other hand, the NCEP ATP-III recommendations for MeSy management include lifestyle changes like diet, rather than pharmacological therapy. Therefore, we focused on analyzing the effect of HSE powder (HSEP) treatment alone or combined with diet on the lipid levels of MeSy patients.

Materials and methods

Study design

A factorial, randomized, follow-up study was designed to evaluate the effect of HSEP on lipid levels in individuals with and without MeSy. The study was carried out at the Early Detection of Metabolic Syndrome Unit on the Health Sciences Campus of the Universidad de Guadalajara, Mexico. The protocol was approved by the Research Committee of the Health Sciences Campus (Universidad de Guadalajara), and all participants provided written informed consent.

Subjects

Volunteers of either sex, ranging in age from 30-71 years, participated in this study. The MeSy and non-MeSy individuals were diagnosed according to NCEP-ATP III criteria. Briefly, individuals were diagnosed as having MeSy when three or more of the following criteria were present: waist circumference at the iliac crest >40 inches (102 cm) for men or >35 inches (88 cm) for women; triglycerides ≥ 150 mg/dl; HDL <40 mg/dl for men and <50 mg/dl for women; blood pressure (BP) >135 mm Hg systolic or >85 mm Hg diastolic; and fasting blood glucose in the range 110-125 mg/dl.

All participants answered questions regarding their age, habits, and clinical and genetic backgrounds. None of the subjects were taking anti-inflammatory drugs or medications that affect metabolism, blood pressure or sympathetic nervous system activity.

The systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured with an aneroid sphygmomanometer (Welch Allyn Tycos, Skaneateles Falls, NY, USA) after a 10-min rest in the sitting position.

Treatments

Subjects in both the MeSy and non-MeSy (control) groups were randomly allocated to the following treatment groups: T1) preventive treatment (diet), T2) HSEP treatment, or T3) diet combined with HSEP treatment. The biochemical evaluation of the groups was performed prior to treatment (day 0) and after one month (day 31). The mean age and male/female ratio were similar between the MeSy and non-MeSy groups. Diet was individually adjusted for each subject in the first group according to the NCEP-ATP III, providing 30% of energy from fat (<7% of saturated fat), 55% from carbohydrates, and 15% from protein, with <200 mg cholesterol/day. Fiber content ranged from 20 to 30 g. The HSEP treatment was comprised of daily oral ingestion of a capsule containing 100 mg HSEP before breakfast, which corresponds to a dosage of 1.42 mg/kg in a person weighing 70 kg. The third treatment group experienced a combination of both treatments (diet and HSEP). The selection of HSEP dose (Ali et al. 2005) was based on previous toxicological studies and the availability of the HSEP, to ensure treatment of the study groups during the whole experimental period.

Extract preparation

Hibiscus sabdariffa (Tepalcatepec variety) was authenticated by Lopez-Muraira I.G., researcher at the Instituto Tecnológico de Tlajomulco Jalisco herbarium, where voucher specimens were stored for future reference (ITTJ-7101). HSEP was prepared by maceration of 500 g of fresh calyces with 7 l of 30% ethanol (v/v) at 20 °C for 7 days. The macerated substance was filtered and the ethanol evaporated with a rotary evaporator at 35 °C. Microencapsulated powder was obtained from the aqueous extract, which was left in a NIRO spray dryer at 190 °C inlet temperature and 80 °C outlet temperature (Gonzalez-Palomares et al. 2009). The samples obtained from the spray drying were weighed and stored in amber-colored vials. The powder yield obtained from the spray drying was approximately 10 g/l extract filtrate with 3% moisture and a pH of 3.4.

Anthocyanin analysis

Before HPLC analysis, HSPE was acid hydrolyzed as described by Zhang et al. (2004). Briefly, 100 mg of HSEP was dispersed in a 1:1 water/methanol solution containing 2 N HCl, syringe-filtered through 0.22 mm nylon membrane (Millipore) and incubated at 100 ± 2 °C for 60 min, immediately cooled to room temperature for analysis. A volume of 25 μl was injected onto a Zorbax C18 (250 mm × 4.6 mm × 5 μm) column. Anthocyanidins were eluted by an isocratic gradient of 50:50:0.01 (v/v) methanol:water:trifluoroacetic acid for 20 min at 1 ml/min using a Thermo Finigan HPLC system. Detection was performed at 522 nm, and spectral data from 490–590 nm were also collected, using a photo-diode array detector (UV6000). Anthocyanidins were identified by comparing the sample retention times and peaks with those of known standards for cyanidin-, and delphinidin- chlorides (Extrasynthese. Lyon, France). Reference standards ranging between 0.2 and 100 mg/l were prepared by dissolving either cyanidin- or delphinidin-chlorides in 1% HCl solution. Linear regression analysis was carried out on the data for peak area versus concentration. A linear calibration was obtained with 98% accuracy. The cyanidin and delphinidin content (mg/g) of the HSPE was calculated using its corresponding standard curve, while the total amount of anthocyanins (192.4 mg per gram of dry base) was obtained indirectly employing the factors 2.07 and 2.15 for delphinidin-sambubioside and cyanidin-sambubioside, these

compounds were found in a ratio of 1.7:1.0, respectively. Both sambubiosides constituted more than 99% of the total anthocyanins.

Metabolic evaluation

At the beginning of the study, blood samples were obtained from the subjects after a 12-h overnight fast. The serum was separated by centrifugation of the freshly drawn blood and immediately processed for the measurement of blood levels of the following biochemical parameters: total cholesterol (TC), triglycerides (TAG), high-density lipoprotein cholesterol (HDL-c), urea, creatinine, alanine amino transferase (ALT), aspartate amino transferase (AST), and glucose. All biochemical measurements were conducted with commercially available kits (Biosystems, Spain) according to the manufacturers' recommendations. The concentration of low-density lipoprotein cholesterol (LDL-c) was calculated using the Friedewald equation (Friedewald et al. 1972). After one month, the same parameters were measured again.

Another important metabolic marker, ratio of triglyceride to high-density lipoprotein cholesterol concentrations (TAG/HDL-c ratio), was also considered. Insulin resistance was defined as a TAG/HDL-c ratio >3.0 (Brehm et al. 2004). Hypercholesterolemia was defined as a TC >200 mg/dl and hypertriglyceridemia as a TAG >150 mg/dl.

Statistical analyses

The student's *t*-test for dependent samples was used to evaluate the change between pre- and post-treatment within the corresponding diagnostic and treatment groups. Inter- and intra-treatment comparisons were evaluated by two way analysis of variance (ANOVA) using the differences between pre- and post-treatment with interactions; being one factor the diagnosis (either control or MeSy) and treatment as the second factor (I: diet, II: HSEP, and III: diet plus HSEP). In addition, a McNemar test for the significance of changes was performed to evaluate the pre- and post-treatment changes in hypercholesterolemia, hypertriglyceridemia, hypertension, and IR with a one-tailed probability. The Statistical Package for the Social Sciences (SPSS for Windows, version 10.0.6, 27 Nov 1999) software was used for all analyses. A p value ≤ 0.05 was considered significant.

Results

The clinical and laboratory analysis was performed in 222 apparently healthy volunteers, 150 of whom did not present evidence of MeSy. From this non-MeSy group, 80 subjects were selected to form the control group. The other 72 subjects were diagnosed with MeSy, which represents a prevalence of 32.4% in the analyzed sample. Among the groups, mean values for age and body mass index and the female: male ratios were homogeneous (49.0 ± 7.2 years, 29.3 ± 4.5 kg/m^2, and 2:1). After one month of treatment, all clinical and biochemical parameters were determined. This phase was only concluded by 51 MeSy patients and 73 controls. The post-treatment evaluation was not completed by 21 MeSy subjects and 7 controls for non-medical reasons; they withdrew their appointment for the post-treatment evaluation. For the analysis of the clinical and biochemical data, only individuals who completed the treatment were considered.

The mean values ($\pm$ SD) of the clinical and parametric values in the pre- and post-treatment phases for controls and MeSy patients are shown in Tables 1 and 2, respectively. In order to analyze if the changes in the clinical and biochemical parameters after treatment were significant, a *t*-test was performed. HSEP treatment reduced glucose (95.1 ± 24.1 mg/dl, $p<0.05$), TC (179.7 ± 21.2 mg/dl, $p<0.05$), and LDL-c (104.1 ± 20.4 mg/dl, $p<0.05$) levels, and increased the HDL-c (44.5 ± 8.3 mg/dl, $p<0.001$) levels in MeSy patients (Table 2). A TAG-lowering post-treatment effect was also demonstrated in all groups, but this reduction was only significant in the control groups treated with diet (90.3 ± 36.5 mg/dl, $p<0.01$) or HSEP (113.8 ± 54.1 mg/dl, $p<0.01$), and MeSy patients treated with HSEP and diet (109.9 ± 46.3 mg/dl, $p<0.001$). The TAG/HDL-c ratio was also significantly reduced by HSEP treatment (2.8 ± 1.5 and 3.5 ± 1.7 in control and MeSy, respectively), meaning an improvement in the IR stage (Tables 1 and 2). In addition, we analyzed some hepatic and renal biochemical parameters. The urea, creatinine, ALT, and AST pre- and post-treatment levels remained within the range of normal values in all treated groups.

Moreover, we analyzed the mean absolute inter- and intra-treatment differences (final – basal treatment values) of all clinically and biochemically described parameters. Inter-treatment differences were only found for the following: TC, HDL-c, and TAG/HDL-c ratio (ANOVA $p=0.019$, 0.002, and 0.025, respectively; Fig. 1). In spite of the inter-treatment difference in TAG values being almost significant (ANOVA $p=0.082$), these data were

Table 1
Mean pre- and post-treatment values of clinical and biochemical variables in the control groups.

	PRE-TREATMENT			**POST-TREATMENT**		
	DIET n=27	**HSEP** n=26	**HSEP+D** n=20	**DIET** n=27	**HSEP** n=26	**HSEP+D** n=20
SBP (mmHg)	119.8 ± 11.2	121.6 ± 12.1	112.8 ± 12.1	117.3 ± 8.9	121.0 ± 7.6	ND
DBP (mmHg)	76.3 ± 11.3	77.5 ± 6.9	81.0 ± 9.0	74.3 ± 7.7	77.8 ± 5.3	ND
Glucose (mg/dl)	95.8 ± 9.6	91.2 ± 10.3	90.6 ± 11.0	**89.9 ± 10.8***	**85.1 ± 8.6***	**82.7 ± 9.5***
Urea (mg/dl)	26.5 ± 8.2	30.9 ± 7.4	28.0 ± 6.1	29.7 ± 5.3	30.9 ± 6.1	30.2 ± 5.2
Creatinine (mg/dl)	0.82 ± 0.27	0.94 ± 0.29	0.78 ± 0.25	0.82 ± 0.22	0.85 ± 0.22	**0.91 ± 0.15***
AST (UI/L)	17.5 ± 9.2	15.3 ± 6.3	14.9 ± 7.0	**8.7 ± 4.3*****	**11.2 ± 5.1***	14.8 ± 5.5
ALT (UI/L)	19.2 ± 13.8	16.6 ± 9.0	14.3 ± 5.5	**8.8 ± 4.7*****	**11.0 ± 6.2***	13.5 ± 6.3
Total cholesterol (mg/dl)	202.9 ± 38.5	202.6 ± 41.6	181.2 ± 24.6	**181.7 ± 29.2*****	197.5 ± 37.0	172.9 ± 33.1
HDL-c (mg/dl)	46.7 ± 9.3	41.6 ± 10.3	41.2 ± 12.4	**47.1 ± 9.1***	**45.8 ± 9.3***	42.8 ± 9.6
LDL-c (mg/dl)	131.3 ± 38.3	124.1 ± 41.7	118.8 ± 22.8	**111.8 ± 28.9****	124.5 ± 35.5	113.9 ± 37.2
VLDL-c (mg/dl)	23.7 ± 11.3	29.6 ± 12.2	20.4 ± 13.2	**18.0 ± 7.3***	**22.7 ± 10.8****	18.7 ± 8.9
Triglycerides (mg/dl)	112.5 ± 39.7	148.0 ± 61.3	102.0 ± 66.3	**90.3 ± 36.5****	**113.8 ± 54.1****	93.6 ± 44.7
TAG/HDL-c ratio	2.5 ± 1.0	3.7 ± 1.7	3.1 ± 3.0	**2.0 ± 0.9***	**2.8 ± 1.5****	2.4 ± 1.4

HSEP, *Hibiscus sabdariffa* extract powder.
Mean value $\pm$ SD; SBP, systolic blood pressure; D, diet; DBP, diastolic blood pressure; TC, total cholesterol; TAG, triglycerides; HDL-c, high-density lipoprotein-cholesterol; LDL-c, low-density lipoprotein-cholesterol; VLDL-c, very low-density lipoprotein-cholesterol; ALT, alanine aminotransferase; AST, aspartate aminotransferase. N.D. not determined. *$p<0.05$, **$p<0.01$, ***$p<0.001$ for a comparison of pre- and post-treatment values in the respective groups using t-test.

Table 2
Mean pre- and post-treatment values of clinical and biochemical variables in the MeSy groups.

	PRE-TREATMENT			**POST-TREATMENT**		
	DIET n=11	**HSEP** n=18	**HSEP+D** n=22	**DIET** n=11	**HSEP** n=18	**HSEP+D** n=22
SBP (mmHg)	122.8 ± 7.6	131.0 ± 11.6	136.4 ± 11.4	111.7 ± 9.1	128.6 ± 11.8	**128.8 ± 10.4***
DBP (mmHg)	74.2 ± 4.6	83.8 ± 9.3	86.2 ± 11.3	72.0 ± 6.9	81.1 ± 7.5	**76.5 ± 11.0***
Glucose (mg/dl)	93.0 ± 14.1	103.8 ± 24.7	105.2 ± 16.3	95.0 ± 9.1	**95.1 ± 24.1***	**92.4 ± 21.2****
Urea (mg/dl)	26.2 ± 7.6	31.5 ± 8.7	29.3 ± 5.6	30.9 ± 11.1	32.4 ± 5.3	31.1 ± 6.2
Creatinine (mg/dl)	0.87 ± 0.14	1.00 ± 0.23	0.93 ± 0.28	0.90 ± 0.15	0.94 ± 0.17	1.03 ± 0.49
AST (UI/L)	17.6 ± 8.0	14.6 ± 6.7	17.1 ± 5.2	7.7 ± 4.5	17.7 ± 8.3	**12.7 ± 7.2***
ALT (UI/L)	13.5 ± 9.8	16.5 ± 5.9	18.0 ± 11.9	8.1 ± 3.3	16.7 ± 8.0	12.4 ± 8.0
Total cholesterol (mg/dl)	183.9 ± 24.7	199.8 ± 40.5	181.7 ± 53.3	196.7 ± 22.9	**179.7 ± 21.2***	183.7 ± 46.5
HDL-c (mg/dl)	39.2 ± 6.8	32.0 ± 5.8	34.6 ± 7.0	**48.4 ± 7.2****	**44.5 ± 8.3*****	**45.0 ± 11.5****
LDL-c (mg/dl)	106.1 ± 22.7	130.1 ± 34.8	110.6 ± 48.7	108.4 ± 21.7	**104.1 ± 20.4***	108.6 ± 56.1
VLDL-c (mg/dl)	38.8 ± 19.2	34.8 ± 21.5	35.4 ± 16.6	31.1 ± 16.0	27.5 ± 12.4	**21.9 ± 9.2*****
Triglycerides (mg/dl)	184.3 ± 83.6	172.3 ± 107.4	177.1 ± 82.9	152.9 ± 69.8	137.6 ± 62.1	**109.9 ± 46.3*****
TAG/HDL-c ratio	5.1 ± 3.3	6.2 ± 5.3	5.2 ± 2.4	3.6 ± 2.4	**3.5 ± 1.7***	**2.7 ± 1.2*****

HSEP, *Hibiscus sabdariffa* extract powder.
Mean value ± SD; SBP, systolic blood pressure; D, diet; DBP, diastolic blood pressure; TC, total cholesterol; TAG, triglycerides; HDL-c, high-density lipoprotein-cholesterol; LDL-c, low-density lipoprotein-cholesterol; VLDL-c, very low-density lipoprotein-cholesterol; ALT, alanine aminotransferase; AST, aspartate aminotransferase. N.D. not determined. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ for a comparison of pre- and post-treatment values in the respective groups using t-test.

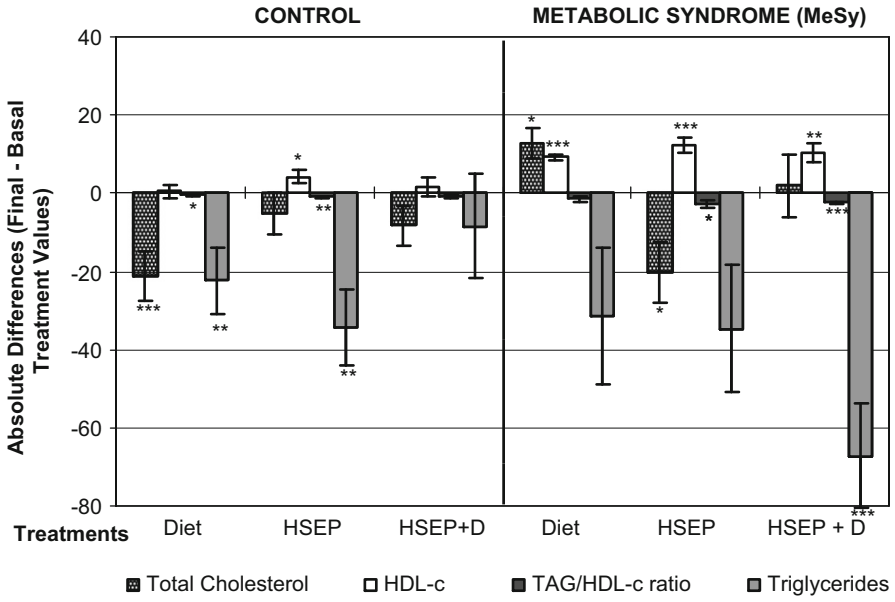

Fig. 1. Absolute differences (Final – Basal treatment values) between 0 and 31 days. Data (mg/dL) are shown as mean ± S.E.M. produced by the treatment with either preventive treatment (D, diet), HSEP or HSEP combined with diet in subjects with and without metabolic syndrome. Intertreatment significant differences were found in total cholesterol, HDL-c and TAG/HDL-c (ANOVA *P*-value < 0.05). Intratreatment significant differences are also shown ($*P \leq 0.05$, $**P \leq 0.01$ and $***P \leq 0.001$ according to *t*-Test). The number of cases of the treated groups are indicated in Tables 1 and 2.

also included due to the importance of TAG in lipid metabolism and MeSy development.

When TC values were analyzed, only the control subjects treated with diet and MeSy patients treated with HSEP presented intra-treatment differences ($p=0.001$ and 0.050, respectively; Fig. 1). Regarding HDL-c, significant intra-treatment differences were found in control subjects treated with HSEP ($p=0.047$) and all MeSy patient treatment groups (Fig. 1). The significant intra-treatment differences in the TAG/HDL-c ratio, an atherogenic index, were observed in control subjects treated with HSEP ($p=0.002$) or diet ($p=0.031$) and MeSy patients treated with HSEP ($p=0.026$) or HSEP plus diet ($p < 0.001$, Fig. 1). Finally, TAG levels were reduced only in control subjects treated with diet or HSEP and MeSy patients treated with HSEP plus diet ($p=0.006$, 0.002, and < 0.001, respectively; Fig. 1). We also measured reductions of 19.7% and 37.8% in the basal TAG levels of MeSy patients treated with HSEP and HSEP plus diet, respectively ($p < 0.001$).

In addition, we analyzed the presence/absence of hypercholesterolemia and hypertriglyceridemia before and after treatment for each treated group using the McNemar Test for the significance of changes. We found a significant reduction in hypercholesterolemic MeSy patients treated with HSEP ($p=0.033$ one-tailed). In the case of hypertriglyceridemia, we found a significant reduction in MeSy patients treated with diet ($p=0.032$, one-tailed) and HSEP combined with diet ($p=0.016$, one-tailed). Nevertheless, we observed that the majority of individuals presenting with either hypercholesterolemia or hypertriglyceridemia had reversed this stage after treatment (data not shown). We performed a similar analysis on hypertension. Almost half of the hypertensive MeSy patients were normotensive after treatment with HSEP plus diet ($p=0.031$, one-tailed). Eight of the sixteen MeSy patients with IR who were treated with HSEP plus diet reverted their TAG/HDL-c ratio to normal values ($p=0.004$, one-tailed).

Discussion

Different studies have demonstrated a lipid-lowering effect of HSE in animal models and clinical trials. This effect has been attributed to anthocyanins, one of the extract's major components (Chen et al. 2003; el-Saadany et al. 1991; Hirunpanich et al. 2006; Mozaffari-Khosravi et al. 2009). Similarly, the effect of HSE on cholesterol and TAG levels in healthy and obese mice was previously evaluated (Alarcon-Aguilar et al. 2007), though they showed only non-significant reductions ($p > 0.05$) in mice treated with 120 mg/kg/day HSE (33.64 mg of total anthocyanins) for 60 days. In contrast, we found a significant reduction (10%) in basal TC levels in MeSy patients ($p < 0.05$) using a total dosage of 100 mg/day of HSEP containing 19.24 mg of anthocyanins during 30 days. This hypocholesterolemic effect is in agreement with reports from Lin et al. (2007) of a mean reduction of 12% in the basal cholesterol levels of subjects with elevated TC levels after 4 weeks of treatment, though with a dosage of 1000 mg/day of lyophilized HSE containing 40.2 mg of anthocyanins. We also found reductions in the basal TAG levels of MeSy patients treated with HSEP and HSEP plus diet. These results are in accordance with the data from Carvajal-Zarrabal et al. (2005) that showed lower TC and TAG levels in animals fed a high cholesterol diet and treated for one week with an ethanol dried HSE. Nevertheless, the authors do not report either anthocyanin content or the chemical profile of the extract.

Although delphidin- and cyanidin-3-sambubiosides were the major anthocyanins identified in our extract, it is necessary to extend and improve upon the chemical profile characterization of HSEP to clarify if there are other compounds that contribute to or enhance the hypocholesterolemic and hypotriglyceridemic effects observed in this study. Moreover, the response to treatment with natural products could be heterogeneous in different models, depending on the concentration and form of the dosage, duration of treatment, and the elaboration and chemical composition of the extract, among other things.

In contrast, we found that both MeSy groups treated with HSEP (HSEP and HSEP+diet) had significantly increased basal HDL-c levels after treatment (37.5% and 29.4%, respectively). These findings are not in accordance with a previous report (Hirunpanich et al. 2006) in which serum HDL-c levels were not affected after *H. sabdariffa* treatment of hypercholesterolemic rats. Interestingly, previous studies have established that a 1 mg/dl increase in basal HDL-c levels reduces the risk of coronary death by 6%. In fact, increases in HDL-c levels have clinical relevance because it has been postulated as an adjunctive therapy to prevent and treat cardiovascular disease (Singh et al. 2007). Besides LDL-c and TAG-lowering strategies, a basal HDL-c level increase is widely recommended in the clinical management of MeSy (ATP III guidelines; Grundy 2005). The increase in basal HDL-c levels could be occurring through lifestyle modifications. In this regard, we corroborated that the preventive treatment represented by diet itself is capable of increasing the HDL-c levels as previously described (Grundy 2005); not only that, but treatment of MeSy patients with HSEP and HSEP plus diet significantly increased the basal HDL-c levels ($p < 0.001$ and 0.003, respectively). Therefore, complementary therapies, such as HSEP consumption, could be considered in the clinical management of MeSy. In addition, reduction of the TAG/HDL-c ratio, an important metabolic marker of IR (McLaughlin et al. 2003), in MeSy patients treated with HSEP or HSEP plus diet supports this recommendation.

In this study, we orally administered 100 mg HSEP per day (1.4 mg/kg) to ensure the biological safety from a toxicological point of view (Onyenekwe et al. 1999, Orisakwe et al. 2004). In addition, we measured the serum levels of hepatic enzymes, urea, and creatinine before and after treatment in all treated subjects. The mean values of these biochemical parameters remained in the normal range, indicating no hepatic or renal toxicity.

Finally, in this study, we focused our investigation on MeSy treatment and hyperlipidemia. In Mexico, there is a high MeSy prevalence compared to industrialized countries (Aguilar-Salinas et al. 2004; Lorenzo et al. 2005; Ramírez-Vargas et al. 2007; Zonana-Nacach and Castillón-Chapa 2006). Metabolic syndrome is a reversible clinical stage of diabetes and cardiovascular disease. Thus, it is important to investigate alternative therapeutic strategies in the management of MeSy, such as natural products like plant extracts. The antihypertensive and hypolipidemic properties of HSE have been demonstrated for hypertensive and diabetic patients, respectively, in clinical trials and *in vivo* experiments (Herrera-Arellano et al. 2004, 2007; Mozaffari-Khosravi et al. 2009). Indeed, we demonstrated a hypotensive effect of HSEP combined with a preventive treatment (diet) in MeSy patients, confirming previous studies (Herrera-Arellano et al. 2004, 2007). It has also been postulated that anthocyanins regulate adipocyte function, having important implications for preventing MeSy (Tsuda 2008). Accordingly, we concluded that HSEP could be a useful complement in MeSy treatment due to its hypolipidemic and hypotensive effects.

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