Cardiovascular and Renal Effects of Hibiscus Sabdariffa Linnaeus. in Patients with Diabetic Nephropathy: A Randomized, Double-Blind, Controlled Trial

Roya Sakhaei; MSc[1,2], Azadeh Nadjarzadeh; PhD[1,2], Akram Esmaeili; MSc[1,2], Mohammad Mohammadi; PhD[3], Roya Hemayati; PhD[4], Javad Zavar Reza; PhD[5], Hassan Mozaffari-Khosravi; PhD[2,6] & Nahid Ramezani-Jolfaie; PhD[3*]

[1] Nutrition and Food Security Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

[2] Department of Nutrition, School of Public Health, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

[3] Department of Community Medicine, School of Medicine, Hormozgan University of Medical Sciences, Bandar Abbas, Iran.

[4] Department of Internal Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

[5] Department of Medical Biochemistry, School of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

[6] Yazd Diabetic Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

ARTICLE INFO

ORIGINAL ARTICLE

ABSTRACT

Background: *Hibiscus sabdariffa linnaeus* (HSL) is a tropical plant with a high content of anthocyanin, traditionally found to have beneficial biological activities. This randomized, double-blind, placebo-controlled, and parallel trial was conducted to assess some renal and cardiovascular effects of supplementation with HSL in patients with diabetic nephropathy. **Methods**: The study protocol was completed by 60 patients (38 females and 22 males) who were randomly assigned into the supplemented (SG) and Placebo groups (PG). The SG was treated with 425 mg of HSL twice daily and the PG received the placebo. Anthropometric and dietary information as well as fasting blood and urine samples were collected at the baseline and end of an 8-week intervention period. **Results**: Compared with the PG, supplementation with HSL significantly reduced systolic blood pressure ($P = 0.01$) and high-sensitivity C-reactive protein ($P = 0.004$). A significant increase was observed in total antioxidant capacity levels in the SG ($P = 0.03$). Moreover, we found a significant reduction in the levels of blood urea nitrogen ($P < 0.001$), blood creatinine ($P = 0.002$), urine creatinine ($P < 0.001$), and urine albumin ($P < 0.001$) in the SG compared with the PG. However, no significant change was observed in diastolic blood pressure, fasting blood glucose, and glomerular filtration rate following intervention between two groups. **Conclusions:** HSL supplementation seems to be beneficial in improving the outcomes of patients with diabetic nephropathy; however, no considerable effect was observed on fasting glucose levels. Large-scale trials are needed to better understand its efficiency and safety for long-term use.

Keywords: *Hibiscus sabdariffa; Diabetic nephropathies; Lipids; Blood pressure.*

Introduction

Diabetes mellitus is a metabolic disorder characterized by increased levels of glucose and lipids. These patients may be susceptible to microvascular defects such as neuropathy, retinopathy, and nephropathy in the long run (Matsui *et al.*, 2007). High blood pressure,

especially glomerular hypertension can exacerbate complications associated with diabetes, including diabetic nephropathy (UK Prospective Diabetes Study Group, 1998, Wilmer *et al.*, 2003). The prevalence of high blood pressure in patients with diabetes is twice as common. The risk of microvascular complications of diabetes reduced by 13% for every 10 mmHg reduction in blood pressure (Gross *et al.*, 2005, Wilmer *et al.*, 2003). Diabetic nephropathy is a chronic and progressive renal disease that affects 20% to 50% of the diabetic patients (Gross *et al.*, 2005, Schena and Gesualdo, 2005). Currently, over 366 million people have diabetes in the world. Among these people, about 183 million individuals are at risk for diabetic nephropathy (Whiting *et al.*, 2011). Microalbuminuria is the first clinical sign of diabetic nephropathy, which if not treated, will gradually change into proteinuria and ultimately lead to renal failure (Gross *et al.*, 2005, Schena and Gesualdo, 2005).

In treating this disease, blood glucose regulators and blood pressure drugs are usually prescribed. In addition, a diet with low content of protein and sodium is commonly recommended for treating diabetic nephropathy (Adler *et al.*, 2000, Federation and Nephrology, 2003, Whiting *et al.*, 2011). The use of medicinal plants is also another therapeutic method for preventing and controlling diabetes complications. For instance, a number of studies found beneficial effects of chamomile (Khan *et al.*, 2014, Zemestani *et al.*, 2016), cinnamon (Azimi *et al.*, 2016, Zare *et al.*, 2018), green tea (Iso *et al.*, 2006), and ginger (Azimi *et al.*, 2016, Jafarnejad *et al.*, 2017) on lipid and glycemic markers, antioxidant status, and diabetic complications in patients with type 2 diabetes.

Hibiscus sabdariffa Linnaeus (HSL) is a tropical plant from the Malvaceae family, which is known by a variety of local names in many different countries (e.g. hibiscus tea, sour tea, sudan tea, roselle, red sorrel, karkad, bissap, rauge oseille, quimbombo chino, etc) (Hirunpanich *et al.*, 2005, Morton, 1987). This plant contains plant acids such as malic, tartaric, and citric acid, polysaccharides made from arabinose, galactose, glucose, and rhamnose, as well as less amounts of galacturonic acid, glucuronic acid, manose, and xylose. It also contains many different anthocyanins such as cyanidin 3-rutinoside, delphinidin 3-sambubioside, cyanidin 3-sambubioside, cyanidin 3-glucoside, and delphinidin 3-glucoside (Ali *et al.*, 2005, Sindi *et al.*, 2014). Some research demonstrated beneficial effects of its extract on health such as antimicrobial, antifungal, and anti-cancer activities, sexually stimulating, appetizing, and energizing (D'Heureux- Calix and Badrie, 2004, Lin *et al.*, 2007, Morton, 1987). It was also used as a blood pressure and cholesterol lowering agent to prevent and treat renal and bladder stones (Hirunpanich *et al.*, 2005, Morton, 1987). HSL may play a beneficial role in preventing chronic diseases such as hypertension, cardiovascular disease, and diabetes (Serban *et al.*, 2015, Sindi *et al.*, 2014). The properties of this plant are attributed to bioactive antioxidants such as polyphenols and flavonoids, and also polysaccharides and organic acids (Ali *et al.*, 2005, Morton, 1987).

Most of the previous clinical trials focused on the investigation of antihypertensive effects of HSL and provided evidence for effectiveness of this plant in reducing blood pressure (Herrera-Arellano *et al.*, 2007, McKay *et al.*, 2010, Mozaffari-Khosravi *et al.*, 2009). Moreover, evidences in animal models show that HSL has the potential to be an adjuvant for diabetic therapy due to its anti-insulin resistance properties and its effect on hypoglycemia, hypolipidemia, and antioxidation (Peng *et al.*, 2011, Yang *et al.*, 2013a). It was also reported that polyphenol extracts from HSL could attenuate nephropathy in diabetic rats (Lee *et al.*, 2009, Yang *et al.*, 2013a). Therefore, considering the beneficial effects of consuming HSL, and lack of interventional studies on the efficacy of this therapeutic plant in patients with diabetic nephropathy, the aim of the present study was to assess the effects of HSL supplementation on renal function and some cardiovascular markers in diabetic nephropathy patients.

Materials and Methods

Study design and participants: A randomized, double-blind, placebo-controlled clinical trial was conducted among patients with diabetic nephropathy who referred to diabetes clinic of Shahid Mohammad Montazeri Hospital in Najafabad, Isfahan, Iran from April until October 2014. Sample size was estimated considering type 1 (α) and type 2 (β) errors of 0.05 and 0.2 (power = 80%), respectively and urinary albumin was considered as a key variable. The standard deviation (SD) of albumin was 0.3 g/dl and the mean difference (d) was 1.5 g/dl according to a former study (Wang *et al.*, 2011). Therefore, the sample size was estimated as 27 participants in each group using the suggested formula for parallel clinical trials. To consider the probable dropouts during the study, we recruited 35 patients in each group. The criteria for entering the study were having: 18 years of age and over; diagnosed diabetic nephropathy (proteinuria with total urinary protein excretion between 30-500 mg per 24 hours in two examinations at an interval of more than 1 month), glycosylated hemoglobin (HbA1c) less than 9%, blood pressure less than 140/90 mmHg, and glomerular filtration rate (GFR) more than 30 mL/min; and taking a constant dosage of renin-angiotensin inhibitor drugs, angiotensin receptor antagonist, and HMG-CoA reductase inhibitors during the past 3 months of the study. Exclusion criteria at recruitment included: 1) taking alcohol drinks, vitamins, minerals, and other nutritional supplements; 2) change in type and dosage of prescribed medications or change in dietary intakes during the past 3 months; 3) unwillingness to continue to participate in the study.

Study intervention: Participants were randomly assigned to the supplemented group (SG) and placebo group (PG) using the random numbers table. All enrolled patients were advised to follow their usual diet and physical activity during the study period, which was 8 weeks. The pills were bought from Barij Essence Pharmaceutical Company, Kashan, Iran (http://barijessence.com). According to the information provided by this company, dried extract of calyces of roselle flowers (HSL) was applied for preparation of supplement pills with total weight of 425 mg (containing active ingredients such as flavonoids, hibiscus acid, and various anthocyanins). Standardization of supplement pills was performed based on the presence of at least 5.56 mg anthocyanin per each pill. The placebo pills contained 425 mg of starch and were identical in shape, size, and package to the supplements. The corresponding supplement and placebo pills were taken twice daily in the morning and in the evening by the SG and PG, respectively. These pills were code numbered and prepared by the manufacturer at the commencement of the study. Moreover, all participants and investigators were unaware of the treatment allocation. After completion of the study, the number of unused pills was counted for each individual and if less than 80% of the administered dosage of HSL was consumed during the intervention, the individual was excluded from the study. Participants were contacted by phone every week to check their compliance with the study procedure, response to questions, and avoid sample loss.

Measurements: The baseline and endpoint data were obtained, including anthropometric measurements, blood pressure, morning urine collection, fasting blood samples, and dietary record.

Body weight was measured by a standard digital weighing scale (SECA, Germany) to the nearest 0.1 kg in light clothing and in a fasted state. Height was measured using wall-mounted stadiometer (to the nearest 0.5 cm) without shoes. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared.

Systolic (SBP) and diastolic blood pressure (DBP) was measured using a digital blood pressure monitor (Riester, Germany) after at least 15 minutes rest, twice with a 10 minutes interval. At last, the mean of two measures was reported.

The outcomes of interest were as follows: levels of fasting blood glucose (FBG), high-sensitivity C-reactive protein (hs-CRP), total antioxidant capacity (TAC), and kidney markers glomerular

filtration rate (GFR), blood urea nitrogen (BUN), blood and urine creatinine (Cr), urine albumin (Alb), 24-hour urine protein, and 24-hour urine volume. Prior to and after the intervention, blood samples for biochemical analysis were obtained from peripheral venous blood after 12-14 h fasting. All samples were centrifuged immediately at 3000 rpm for 10 min. The separated sera were placed in 1-mL microtubes and stored at -80 °C until analysis. Morning urine samples were also collected in sterilized bottles and kept at -20 °C until testing. Urine albumin concentration was determined by ELISA, immunoabsorbant assay. All tests were performed only in a laboratory to prevent measurement error.

Dietary intakes of energy as well as macro- and micronutrients were estimated by a three-day food record at the beginning and end of the study. A trained nutritionist analyzed dietary data using Nutritionist IV software (First Databank, San Bruno, CA, USA).

Ethical considerations: Written consent was obtained from patients who met the inclusion criteria. The present study was registered in Iranian Registry of Clinical Trials (www.irct.ir; IRCT code: IRCT2014041510826N8). The ethical approval of the study protocol was granted by the Ethics Committee of Shahid Sadoughi University of Medical Sciences, Yazd, Iran (ethics registration number: 172748)

Data analysis: All statistical calculations were performed using SPSS, version 16.0 (SPSS Inc., Chicago, IL). Data were presented as means ± standard deviations (SDs). A probability value of 0.05 was defined statistically significant. Chi Square test was run to compare qualitative variables between the two groups. Considering the normal distribution of quantitative variables based on the Kolmogorov-Smirnov test, we used paired samples *t-tests* to evaluate within-group treatment effects, and independent sample *t-test* to evaluate between-group treatment effects. If the baseline values were significantly different, we performed analyses by adjusting for the baseline values using univariate analysis of covariance (ANCOVA).

Results

As shown in **Figure 1,** 60 participants (SG, n = 30; PG, n = 30) completed the entire study protocol. The participants' mean age was 52 years and females constituted 63% of the study sample. The mean (±SD) of diabetes duration were 18 ± 5.01 and 16 ± 7.24 years in the SG and PG, respectively. The majority of participants (80%) had insulin injection and others continued to use the blood glucose lowering drugs prescribed prior to the study. **Table 1** summarizes baseline demographic and clinical characteristics by treatment type and no significant difference was observed in these variables between the study groups. No considerable change was found in weight and BMI values between groups at the baseline and after 8 weeks of intervention ($P > 0.05$).

The mean dietary intake of each group at the baseline and endpoint is shown in **Table 2**. No significant changes were observed in macro- and micronutrient intake in the treatment groups. No significant differences were found between the groups either at baseline or at the end of the study.

The results regarding the effect of supplementation with HSL on renal function are shown in **Table 3**. The mean change of GFR did not differ statistically from the baseline in both groups and significant difference was found between the supplement and placebo groups. However, BUN levels decreased significantly in the SG compared to baseline of the intervention (33.5 ± 25.22 vs. 24.25 ± 17.65 mg/dl), while an incremental change was observed in the PG (24.9 ± 16.6 vs. 27.04 ± 15.98 mg/dl). A significant reduction was found in blood creatinine by -0.27 g/dl and urine creatinine by -192.75 g/dl in the SG; however, no significant differences was observed in the PG. At the end of the eighth week, the concentration of urine albumin decreased after HSL supplementation significantly; however, no significant change was observed in the PG. The mean changes were observed from baseline to eighth week in urine albumin concentration between the two groups (SG: -20.56 ± 14.35; PG: -12.61 ± 17.02; $P < 0.05$). Although a significant reduction was found in 24-hour urine protein levels

in the SG, this change was not significantly different from the PG. Moreover, a significant increase was seen in 24-hour urine volume in the SG (372.5 ± 302.3) compared with the PG (-61.9 ± 270.6).

As shown in **Table 4**, no significant difference was found in FBG concentrations within each group and between the two groups during the study period. The mean change of SBP were statistically significant in the SG (-15.5 ± 6.04 mmHg) compared with the mean change in the PG (-8.09 ±

10.7 mmHg). Although we observed a beneficial effect of HSL supplementation on reducing DBP, this change was not statistically significant ($P = 0.06$). Moreover, a significant increase was found in mean change of TAC levels (11.6 ± 21.96) and a significant decrease was observed in mean change of hs-CRP levels (-0.6 ± 0.71 mg/l) in the SG after 8 weeks of supplementation. In comparing the effect of HSL and placebo, we found a significant reducing effect of this supplement on hs-CRP levels ($P = 0.004$).
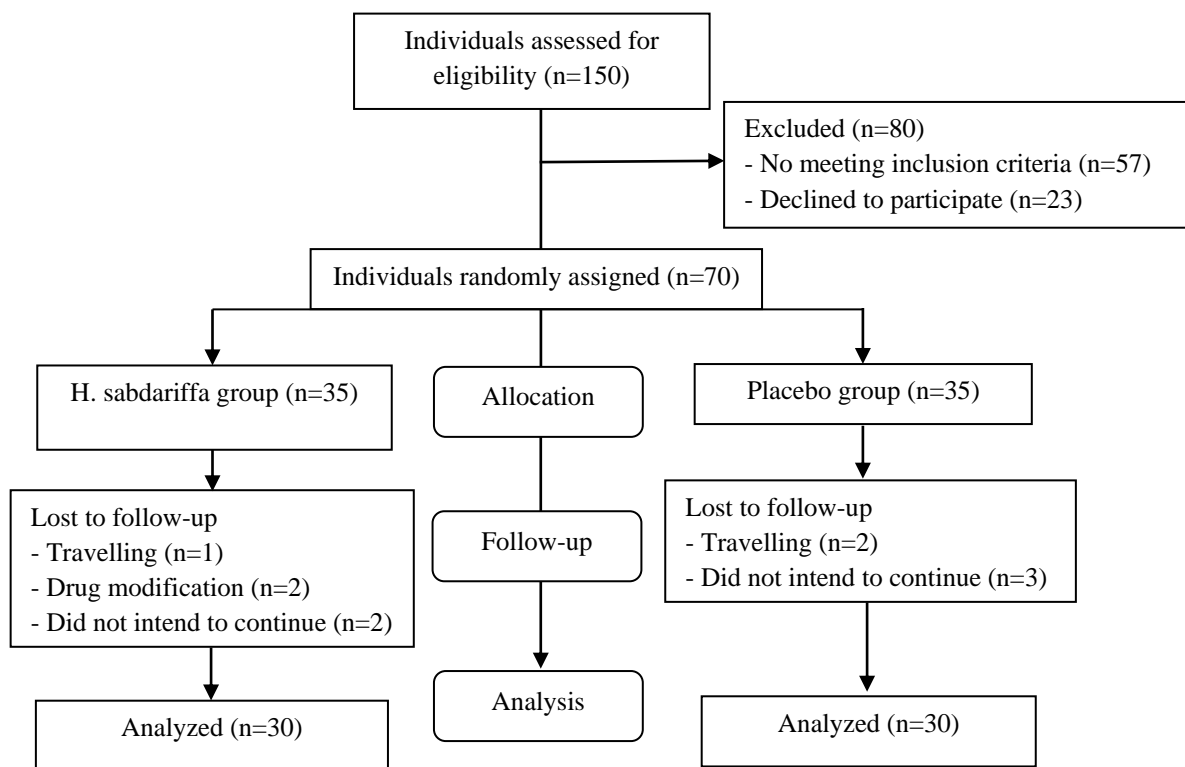
Figure 1. Flowchart of patient enrollment for the randomized placebo-controlled trial of *Hibiscus sabdariffa Linn.* supplementation in patients with diabetic nephropathy

Table 1. Comparison of baseline demographic and clinical characteristics between the Hibiscus sabdariffa supplemented group (SG) and placebo group (PG)

Variables	SG (n = 30)	PG (n = 30)	P-value
Quantitative variables	Mean ± SD	Mean ± SD	
Age (y)	52.00 ± 1.05	52.00 ± 7.20	0.46[a]
Weight (kg)			
Before	83.82 ± 18.08	83.67 ± 14.71	0.97[a]
After	84.48 ± 19.69	84.27 ± 14.74	0.98[a]
Body mass index (kg/m^2)			
Before	31.42 ± 5.1	31.74 ± 5.93	0.86[a]

Table 1. Comparison of baseline demographic and clinical characteristics between the Hibiscus sabdariffa supplemented group (SG) and placebo group (PG)

Variables	SG (n = 30)	PG (n = 30)	P-value
After	31.64 ± 5.55	31.99 ± 6.03	0.85[a]
Duration of diabetes (y)	18.00 ± 5.01	16.00 ± 7.24	0.26[a]
Qualitative variables	N (%)	N (%)	
Gender			0.21[b]
Male	12 (40)	10 (33.3)	
Female	18 (60)	20 (66.7)	
Use of smoke			
Non-smoker	15 (75)	17 (81)	0.52[b]
Smoker	5 (25)	4 (19)	0.35[b]
Use of blood pressure-lowering drugs			
Yes	18 (90)	19 (90.5)	0.43[b]
No	2 (10)	2 (9.5)	0.22[b]
Control of diabetes			
Insulin injection	14 (70)	19 (90.5)	0.30[b]
Blood glucose lowering drugs	6 (30)	2 (9.5)	0.33[b]

[a]: Independent samples t-test ; [b]: Chi Square test.

Table 2. Dietary intakes at baseline and at the endpoint by Hibiscus sabdariffa supplemented group (SG) and placebo group (PG)

Variables/groups	Time of study		P-value[a]
	Baseline	Endpoint	
Energy (kcal/d)			
SG (n = 30)	1598 ± 510[b]	1600 ± 511	0.36
PG (n = 30)	1586 ± 340	1595 ± 510	0.60
Protein (g/d)			
SG	64 ± 22	65 ± 24	0.58
PG	61 ± 23	61 ± 21	0.47
Carbohydrate (g/d)			
SG	215 ± 52	216 ± 54	0.18
PG	220 ± 53	223 ± 52	0.08
Fiber (g/d)			
SG	12 ± 2	12 ± 3	0.25
PG	13 ± 3	13 ± 4	0.07
Fat (g/d)			
SG	51 ± 18	51 ± 17	0.66
PG	50 ± 16	50 ± 15	0.59
Cholesterol (mg/d)			
SG	200 ± 135	177 ± 140	0.17
PG	170 ± 166	188 ± 179	0.15
Vitamin E (mg/d)			
SG	8 ± 6	9 ± 6	0.33
PG	7 ± 5	7 ± 4	0.40
Vitamin C (mg/d)			
SG	77 ± 44.5	78 ± 44.5	0.28
PG	78 ± 34	77 ± 34	0.36
Fluid intake (ml/d)			
SG	1885 ± 255	1830 ± 195	0.93
PG	1950 ± 300	1915 ± 269	0.23

[a]: Paired *t-test*; [b]: Mean ± SD

Table 3. Comparison of renal function variables within and between the Hibiscus sabdariffa supplemented group (SG) and placebo group (PG)

Variables	SG (n = 30)	PG (n = 30)	P- value[b]
Glomerular filtration rate (ml/min)			
Before	65.66 ± 59.89[c]	57.66 ± 22.67	0.58
After	54.62 ± 29.19	56.65 ± 81.32	0.79
P-value[a]	0.20	0.75	
Change	-11.02 ± 36.57	-1.00 ± 14.44	0.26
Blood urea nitrogen (mg/dl)			
Before	33.50 ± 5.22	24.90 ± 6.60	0.20
After	24.25 ± 7.65	27.04 ± 5.98	0.59
P-value	0.001	0.08	
Change	-9.30 ± 5.10	2.14 ± 5.34	<0.001
Blood creatinine (g/dl)			
Before	1.37 ± 0.76	1.29 ± 0.78	0.75
After	1.09 ± 0.48	1.37 ± 0.94	0.24
P-value	0.003	0.25	
Change	-0.27 ± 0.36	0.08 ± 0.31	0.002
Urine creatinine (g/dl)			
Before	934.75 ± 340.32	931.47 ± 318.32	0.97
After	742.00 ± 260.24	918.09 ± 283.27	0.04
P-value	<0.001	0.38	
Change	-192.75 ± 112.52	-13.38 ± 69.02	<0.001
Urine albumin (mg/l)			
Before	75.40 ± 26.16	93.04 ± 44.00	0.17
After	54.08 ± 17.54	80.42 ± 40.36	0.03
P-value	<0.001	0.24	
Change	-20.56 ± 14.35	-12.61 ± 17.02	<0.001
24-h urine protein (mg/l)			
Before	361.00 ± 307.04	817.47 ± 535.46	0.002
After	261.00 ± 246.55	745.06 ± 483.34	0.04
P-value	0.004	0.12	
Change	-99.10 ± 170.98	-43.80 ± 169.93	0.30
24-h urine volume (ml)			
Before	1942.50 ± 826.73	1723.80 ± 614.73	0.34
After	2351.00 ± 970.77	1661.90 ± 515.00	0.01
P-value	<0.001	0.30	
Change	372.5 ± 302.39	-61.90 ± 270.6	<0.001

[a]: Paired *t-test;* [b]*:* Student *t-test;* [c]: Mean ± SD

Table 4. Comparison of fasting blood glucose, blood pressure, antioxidant and inflammatory markers within and between the Hibiscus sabdariffa supplemented group (SG) and placebo group (PG)

Variables	SP (n = 30)	PG (n = 30)	P-value[b]
Fasting blood glucose (mg/dl)			
Before	186.35 ± 74.39[c]	154.38 ± 48.86	0.11
After	186.50 ± 70.79	158.66 ± 60.37	0.26
P-value[a]	0.98	0.70	
Change	0.15 ± 45.73	4.28 ± 51.74	0.78
Systolic blood pressure (mmHg)			
Before	135.00 ± 6.80	134 .00± 7.40	0.74
After	119.50 ± 6.04	126.00 ± 9.20	0.01
P-value	<0.001	0.005	
Change	-15.50 ± 6.04	-8.09 ± 10.70	0.07

Table 4. Comparison of fasting blood glucose, blood pressure, antioxidant and inflammatory markers within and between the Hibiscus sabdariffa supplemented group (SG) and placebo group (PG)

Variables	SP (n = 30)	PG (n = 30)	P-value[b]
Diastolic blood pressure (mmHg)			
Before	77.50 ± 4.44	78.09 ± 6.01	0.72
After	75.00 ± 6.04	76.19 ± 4.97	0.45
P-value	0.05	0.25	
Change	-2.50 ± 5.50	-1.90 ± 7.49	0.74
Total antioxidant capacity (µmol/l)			
Before	23.08 ± 21.42	25.30 ± 20.08	0.73
After	34.15 ± 18.98	32.73 ± 16.41	0.80
P-value	0.003	0.23	
Change	11.60 ± 21.96	7.43 ± 26.96	0.63
High-sensitivity C-reactive protein (µmol/l)			
Before	3.12 ± 1.07	3.51 ± 1.14	0.25
After	2.52 ± 0.74	3.63 ± 1.18	<0.001
P-value	0.04	0.25	
Change	-0.60 ± 0.71	-0.11 ± 0.79	0.04

[a]: Paired *t-test;* [b]: Student *t-test;* [c]: Mean ± SD

Discussion

To the best of our knowledge, the cardiovascular and renal effects of HSL supplementation in patients with diabetic nephropathy were not assessed in the past. Findings of the present study showed the beneficial effect of 8 weeks supplementation with HSL on renal markers (reduction of BUN and urine albumin levels) as well as blood and urine creatinine concentrations. We also observed that SBP decreased significantly in patients who received supplement compared with the placebo group. However, reduction of DBP in the supplement group was not statistically significant. Moreover, a significant reducing effect was found on hs-CRP levels and an increasing effect was seen on TAC levels in our study. It should be noted that this supplementation did not affect blood glucose levels.

Our findings over the blood pressure-lowering effect of HSL are in accordance with a previous meta-analysis reporting the beneficial effect of this plant in reducing blood pressure. The health status of participants enrolled in the included studies in this meta-analysis was as follows: healthy participants, patients with metabolic syndrome or type 2 diabetes, and patients with hypertension (Serban *et al.*, 2015). Even though several studies indicated the effects of HSL on reducing blood pressure, no exact mechanism has yet been proposed in this regard (Serban *et al.*, 2015). The vasodilator role of HSL was observed in the animal models, which are probably mediated through the endothelium-derived nitric oxide-cyclic guanosine monophosphate relaxant pathway and also inhibition of calcium influx into vascular smooth muscle cells. Moreover, the blood pressure lowering effect can be attributed to the polyphenolic antioxidants present in this plant, which modulate dilatation of vessels by regulating nitric oxide bioavailability (Ajay *et al.*, 2007, Kearney *et al.*, 2004). It was also claimed that antihypertensive effectiveness of HSL is related to the presence of anthocyanins, which plays a role in antihypertensive mechanisms such as inhibited production of angiotensine I and angiotensine II converting enzyme (Herrera-Arellano *et al.*, 2004, Meunier *et al.*, 1987).

Another finding of this study included the reduction of albuminuria, which can be probably explained by positive changes in oxidative stress and inflammatory markers after intake of HSL or its compounds (Lin *et al.*, 2011, Yamagishi and Imaizumi, 2005). Moreover, a significant increase was found in TAC levels and a significant decrease

was observed in hs-CRP levels in the findings of this study. Some evidence from in vitro studies has shown that the major bioactive compounds of HSL with antioxidant properties including protocatechuic acid, catechin, and epigallocatechin gallate can play roles in scavenging harmful free radicals and regenerating other antioxidants to prevention of cellular oxidative damages (Lin *et al.*, 2005). On the one hand, quercetin, kaempferol, and chlorogenic acid, as major constituents of HSL had promising anti-inflammatory activities (Zhen *et al.*, 2016). On the other hand, the decrease in albuminuria may be due to the effect of this plant on reducing blood pressure, which can prevent kidney damage and result in decreased albuminuria (Noori *et al.*, 2010).

Our finding is in accordance with previous experimental studies in animal models that showed improvement in albuminuria and creatinine clearance as well as reduction in oxidative stress and renal tissue malondialdehyde levels (Wang *et al.*, 2011, Yang *et al.*, 2013a, Yang *et al.*, 2013b). The histological findings in these studies also discovered that polyphenol extracts of HSL could prevent filament loss, restore the tubular outline, and increase cell junctions in renal tubular cells and expression of type IV collagen in diabetic kidneys. Indeed, attenuating renal epithelial mesenchymal transition may result in improved diabetic nephropathy and administration of different forms of HSL or its compounds can be potential adjuvant therapeutic strategies for diabetic nephropathy (Yang *et al.*, 2013a, Yang *et al.*, 2013b).

Our results should be considered in the context of some limitations including the small number of participants and short treatment period. Indeed, the observed changes after a 2-month supplementation with HSL cannot necessarily be reflected the long-term effects. In addition, in our study, HSL supplementation was applied in the form of supplement pills that can have different effects compared with this plant in the form of drinks. Moreover, the measurement of outcomes was only performed at the baseline and end of the follow-up period and serial assessments were not done during the study period. We also assessed the patients' compliance through telephone calls every week and pill count during the entire study period and could not use more precise methods such as assessing the levels of anthocyanins in blood due to budget constraints and lack of available facilities. It should be also mentioned that since the exact mechanisms of the blood pressure and albuminuria lowering effects of HSL are not yet clearly understood, this product should be administered with caution. The randomized double-blind placebo-controlled design was the major strength of the present study.

Consequently, 8 weeks of HSL supplementation in patients with diabetic nephropathy improved renal function and reduced blood pressure, suggesting that this would help to slow progression of diabetic nephropathy and can be recommended for these patients as an herbal drug. However, this supplement did not affect blood glucose levels. So, large-scale trials with longer periods and higher doses of HSL are recommended to better understand its efficiency and safety for long-term use.

Acknowledgments

Authors' contributions

Nadjarzadeh A, Hemayati R, Zavar Reza J, and Mozaffari-Khosravi H designed the research; Esmaeili A conducted the study and collected data; Nadjarzadeh A performed statistical analyses; Sakhaei R, Ramezani-Jolfaie N, and Mohammadi M interpreted data and drafted the manuscript; and all authors read and approved the final manuscript.

Conflict of interest

There was no conflict of interest for the authors of this article. Barij Essence Pharmaceutical Company did not provide any financial or scientific support for this study.

References

Adler AI, et al. 2000. Association of systolic blood pressure with macrovascular and microvascular complications of type 2 diabetes (UKPDS 36): prospective observational study. *British medical jounal.* **321 (7258)**: 412-419.

Ajay M, Chai H, Mustafa A, Gilani AH & Mustafa MR 2007. Mechanisms of the anti-hypertensive effect of Hibiscus sabdariffa L. calyces. *Journal of ethnopharmacology.* **109 (3)**: 388-393.

Ali B, Al Wabel N & Blunden G 2005. Phytochemical, pharmacological and toxicological aspects of Hibiscus sabdariffa L.: a review. *Phytotherapy research.* **19 (5)**: 369-375.

Azimi P, et al. 2016. Effect of cinnamon, cardamom, saffron and ginger consumption on blood pressure and a marker of endothelial function in patients with type 2 diabetes mellitus: A randomized controlled clinical trial. *Blood pressure.* **25 (3)**: 133-140.

D'Heureux- Calix F & Badrie N 2004. Consumer acceptance and physicochemical quality of processed red sorrel/roselle (Hibiscus sabdariffa L.) sauces from enzymatic extracted calyces. *Food service technology.* **4 (4)**: 141-148.

Federation ID & Nephrology ISo 2003. Diabetes and Kidney Disease: Time to Act.

Gross JL, et al. 2005. Diabetic nephropathy: diagnosis, prevention, and treatment. *Diabetes care.* **28 (1)**: 164-176.

Herrera-Arellano A, Flores-Romero S, Chavez-Soto M & Tortoriello J 2004. Effectiveness and tolerability of a standardized extract from Hibiscus sabdariffa in patients with mild to moderate hypertension: a controlled and randomized clinical trial. *Phytomedicine.* **11 (5)**: 375-382.

Herrera-Arellano A, et al. 2007. Clinical effects produced by a standardized herbal medicinal product of Hibiscus sabdariffa on patients with hypertension. A randomized, double-blind, lisinopril-controlled clinical trial. *Planta medica.* **73 (01)**: 6-12.

Hirunpanich V, et al. 2005. Antioxidant Effects of Aqueous Extracts from Dried Calyx of Hibiscus sabdariffa L INN.(Roselle) in Vitro Using Rat Low-Density Lipoprotein (LDL). *Biological and pharmaceutical bulletin.* **28 (3)**: 481-484.

Iso H, Date C, Wakai K, Fukui M & Tamakoshi A 2006. The relationship between green tea and total caffeine intake and risk for self-reported type 2 diabetes among Japanese adults. *Annals of internal medicine.* **144 (8)**: 554-562.

Jafarnejad S, et al. 2017. Effect of ginger (Zingiber officinale) on blood glucose and lipid concentrations in diabetic and hyperlipidemic subjects: A meta-analysis of randomized controlled trials. *Journal of functional foods.* **29**: 127-134.

Kearney PM, Whelton M, Reynolds K, Whelton PK & He J 2004. Worldwide prevalence of hypertension: a systematic review. *Journal of hypertension.* **22 (1)**: 11-19.

Khan SS, Najam R, Anser H, Riaz B & Alam N 2014. Chamomile tea: herbal hypoglycemic alternative for conventional medicine. *Pakistan journal of pharmaceutical sciences.* **27 (5)**: 1509-1514.

Lee WC, et al. 2009. Polyphenol extracts from Hibiscus sabdariffa Linnaeus attenuate nephropathy in experimental type 1 diabetes. *Journal of agricultural and food chemistry.* **57 (6)**: 2206-2210.

Lin C-Y, Tsai S-J, Huang C-S & Yin M-C 2011. Antiglycative effects of protocatechuic acid in the kidneys of diabetic mice. *Journal of agricultural and food chemistry.* **59 (9)**: 5117-5124.

Lin HH, Huang HP, Huang CC, Chen JH & Wang CJ 2005. Hibiscus polyphenol-rich extract induces apoptosis in human gastric carcinoma cells via p53 phosphorylation and p38 MAPK/FasL cascade pathway. *Molecular carcinogenesis.* **43 (2)**: 86-99.

Lin T-L, et al. 2007. Hibiscus sabdariffa extract reduces serum cholesterol in men and women. *Nutrition research.* **27 (3)**: 140-145.

Matsui T, et al. 2007. α-Glucosidase inhibitory profile of catechins and theaflavins. *Journal of agricultural and food chemistry.* **55 (1)**: 99-105.

McKay DL, Chen CY, Saltzman E & Blumberg JB 2010. Hibiscus sabdariffa L. tea (tisane)

lowers blood pressure in prehypertensive and mildly hypertensive adults. *Journal of nutriyion.* **140 (2)**: 298-303.

Meunier M-T, Villié F, Jonadet M, Bastide J & Bastide P 1987. Inhibition of angiotensin I converting enzyme by flavanolic compounds: in vitro and in vivo studies. *Planta medica.* **53 (01)**: 12-15.

Morton J 1987. In: Fruits of warm climates.

Mozaffari-Khosravi H, Jalali-Khanabadi B, Afkhami-Ardekani M, Fatehi F & Noori-Shadkam M 2009. The effects of sour tea (Hibiscus sabdariffa) on hypertension in patients with type II diabetes. *Journal of human hypertension.* **23 (1)**: 48-54.

Noori N, Tabibi H, Hosseinpanah F, Hedayati M & Nafar M 2010. Effects of combined administration of Lipoic Acid and Pyridoxine on serum systemic and vascular inflammatory factors in patients with diabetic nephropathy. *Iranian journal of endocrinology and metabolism.* **12 (2)**: 99-110.

Peng CH, et al. 2011. Hibiscus sabdariffa polyphenolic extract inhibits hyperglycemia, hyperlipidemia, and glycation-oxidative stress while improving insulin resistance. *Journal of agricultural and food chemistry.* **59 (18)**: 9901-9909.

Schena FP & Gesualdo L 2005. Pathogenetic mechanisms of diabetic nephropathy. *Journal of the American society of nephrology.* **16 (3 suppl 1)**: S30-S33.

Serban C, Sahebkar A, Ursoniu S, Andrica F & Banach M 2015. Effect of sour tea (Hibiscus sabdariffa L.) on arterial hypertension: a systematic review and meta-analysis of randomized controlled trials. *Journal of hypertension.* **33 (6)**: 1119-1127.

Sindi HA, Marshall LJ & Morgan MR 2014. Comparative chemical and biochemical analysis of extracts of Hibiscus sabdariffa. *Food Chemistry.* **164**: 23-29.

UK Prospective Diabetes Study Group 1998. Tight blood pressure control and risk of macrovascular and microvascular complications in type 2 diabetes: UKPDS 38. *British medical journal.* **317 (7160)**: 703.

Wang S-C, et al. 2011. Aqueous extract from Hibiscus sabdariffa Linnaeus ameliorate diabetic nephropathy via regulating oxidative status and Akt/Bad/14-3-3γ in an experimental animal model. *Evidence-based complementary and alternative medicine.* **2011**.

Whiting DR, Guariguata L, Weil C & Shaw J 2011. IDF diabetes atlas: global estimates of the prevalence of diabetes for 2011 and 2030. *Diabetes research and clinical practice.* **94 (3)**: 311-321.

Wilmer WA, et al. 2003. Management of glomerular proteinuria: a commentary. *Journal of the American society of nephrology.* **14 (12)**: 3217-3232.

Yamagishi S-i & Imaizumi T 2005. Diabetic vascular complications: pathophysiology, biochemical basis and potential therapeutic strategy. *Current pharmaceutical design.* **11 (18)**: 2279-2299.

Yang Y-S, et al. 2013a. Polyphenols of Hibiscus sabdariffa improved diabetic nephropathy via regulating the pathogenic markers and kidney functions of type 2 diabetic rats. *Journal of functional foods.* **5 (2)**: 810-819.

Yang YS, et al. 2013b. Polyphenols of Hibiscus sabdariffa improved diabetic nephropathy via attenuating renal epithelial mesenchymal transition. *Journal of agricultural and food chemistry.* **61 (31)**: 7545-7551.

Zare R, Nadjarzadeh A, Zarshenas MM, Shams M & Heydari M 2018. Efficacy of cinnamon in patients with type II diabetes mellitus: A randomized controlled clinical trial. *Clinical nutrition.*

Zemestani M, Rafraf M & Asghari-Jafarabadi M 2016. Chamomile tea improves glycemic indices and antioxidants status in patients with type 2 diabetes mellitus. *Nutrition.* **32 (1)**: 66-72.

Zhen J, et al. 2016. Phytochemistry, antioxidant capacity, total phenolic content and anti-inflammatory activity of Hibiscus sabdariffa leaves. *Food chemistry.* **190**: 673-680.