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Metabolic Response to Mulberry Extract Supplementation in Patients With Diabetic Nephropathy

A Randomized Controlled Trial

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Introduction. This study aimed to evaluate the effects of mulberry extract administration on markers of insulin metabolism, lipid concentrations, and biomarkers of inflammation and oxidative stress in patients with diabetic nephropathy (DN).

Materials and Methods. Sixty patients were randomly allocated into 2 groups to receive either 300 mg/d of mulberry extract (n = 30) or placebo (n = 30), twice per day for 12 weeks. Fasting blood samples were taken at the onset of the study and 12 weeks after supplementation to examine markers of insulin metabolism, lipid concentrations, and biomarkers of inflammation and oxidative stress.

Results. Mulberry extract, compared to placebo, resulted in significant reductions in serum triglycerides (-37.3 ± 64.7 mg/dL versus 3.0 ± 78.8 mg/dL, $P = .03$) and very low-density lipoprotein cholesterol (-7.4 ± 12.9 mg/dL versus 0.6 ± 15.8 mg/dL, $P = .03$), and a significant increase in high-density lipoprotein cholesterol concentration (0.5 ± 4.0 mg/dL versus -2.0 ± 5.0 mg/dL, $P = .03$). Other significant changes were in serum high-sensitivity C-reaction protein (-2.3 ± 4.5 µg/mL versus -0.1 ± 2.2 µg/mL, $P = .02$), plasma glutathione (87.8 ± 159.7 µmol/L versus -24.2 ± 138.8 µmol/L, $P = .005$) and malondialdehyde (-0.03 ± 0.5 µmol/L versus 0.7 ± 1.0 µmol/L, $P < .001$).

Conclusions. These findings showed that mulberry extract administration had favorable effects on serum lipids, HSCRP, glutathione, and malondialdehyde levels in DN patients; however, it did not affect markers of insulin metabolism or biomarkers of inflammation and oxidative stress.

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INTRODUCTION

Diabetic nephropathy (DN) is the most common cause of end-stage renal disease,[1] which is associated with macrovascular complications and increased cardiovascular mortality.[2] It affects 25% to 40% of type 1 and type 2 diabetic patients.[3] Both observational studies and randomized controlled trials have demonstrated the central role of hyperglycemia and insulin resistance and its attendant metabolic consequences in the initiation and progression of DN.[4] In addition, previous studies have indicated that inflammatory cytokines and oxidative stress resulted from inflammatory responses induced by hyperglycemia are associated

with the development and progression of DN.[5,6]

For decades, the bioactivity and pharmacological effects of the mulberry leaves have been widely assessed, and it was found that the mulberry leaves had various biological functions such as hypoglycemic,[7] hypolipidemic,[8] anti-inflammatory, antioxidant, and anti-cancer[9] properties. Trimarco and colleagues[8] observed that taking a new combination of nutraceuticals with mulberry resulted in a marked improvement in total cholesterol and low-density lipoprotein cholesterol (LDLC) levels and parameters of glucose homeostasis among dyslipidemic individuals for 4 weeks, but did not influence other lipid parameters. In addition, supplementation with mulberry leaf powder (280 mg mulberry leaf powder, 3 times per day) had potential to decrease serum triglycerides, LDLC, and C-reactive protein (CRP) levels in patients with mild dyslipidemia, for 12 weeks.[10] However, ingestion of mulberry leaf extract with enriched 1-deoxynojirimycin content did not affect fasting plasma glucose (FPG), glycated hemoglobin, or glycated albumin concentrations in individuals with impaired glucose metabolism.[11]

The favorable effects of mulberry supplementations on insulin resistance, biomarkers of inflammation, and oxidative stress may be mediated by its impact on stimulated skeletal muscle 5′-adenosine monophosphate-activated protein kinase activity acutely,[12] scavenging free radicals, inhibiting oxidation, and reducing atherogenic risk.[13] As there is evidence that mulberry intake may decrease insulin resistance as well as has anti-inflammatory and antioxidant effects, we hypothesized a beneficial effect of mulberry extract supplementation on metabolic status in patients with DN. This clinical trial was to examine the effects of mulberry extract administration on glycaemic control, lipid concentrations, biomarkers of inflammation, and oxidative stress among DN patients.

MATERIALS AND METHODS

Trial Design

This was a 12-week randomized, double-blind, placebo-controlled clinical trial (registration number: IRCT2016021512438N18, http://www.irct.ir:).

Participants

The study was conducted among 60 patients with DN aged 45 to 85 years old referred to Akhavan Clinic in Kashan, Iran, from April 2016 to June 2016. We defined DN as diabetic kidney disease with a proteinuria level greater than 0.3 g/24 h, with or without elevation of serum creatinine levels.[1] The exclusion criteria were as follows: the intake of mulberry extract supplements within the past 3 months, uncontrolled diabetes mellitus, current use of warfarin or any anticoagulant agents, malignancy, and liver cirrhosis.

Ethics Statements

The present study was conducted in accordance with the Declaration of Helsinki and an informed consent form was signed by each participates. The study protocol was approved by the ethics committee of Kashan University of Medical Sciences.

Study Design

All participants were matched according to sex, type of medications, BMI, and age, and were then randomly divided into 2 groups to receive either mulberry extract (n = 30: 23 women and 7 men) or placebo (n = 30, 23 women and 7 men) for 12 weeks. The participants were requested not to change their regular physical activity and not to take any nutritional supplements during the 12-week trial. All of the patients completed 3-day food records and 3 physical activity records at the study baseline and weeks 3, 6, and 9 of the intervention, as well as the end of the trial.

Daily macro- and micro-nutrient intakes were analyzed by a nutritionist IV software (First Databank, San Bruno, CA). Physical activity was described as metabolic equivalents (METs) in hours per day. To determine the METs for each patient, we multiplied the times (in hour per day) reported for each physical activity by its related METs coefficient by standard tables.[14]

Intervention

In the treatment group, participants received 300 mg of mulberry extract capsule daily for 12 weeks. Mulberry extract supplements were produced by Barij Essence Pharmaceutical Company (Kashan, Iran), approved by Food and Drug Administration.

Treatment Adherence

Every 4 weeks, the participants were taken enough supplements and placebos to last 3 days

after their next scheduled visit and were instructed to return all unused supplements and placebos at each visit. The remaining supplements and placebos were counted and subtracted from the number provided to determine the number taken. To increase the compliance, all of the participants were receiving short messages on their cell phones to take supplements and placebos every day.

Assessment of Anthropometric Measures

Weight and height of the participants were determined in an overnight fasting status using a standard scale (Seca, Hamburg, Germany) at the onset of the study and after the 12-weeks' treatment. Body mass index was calculated as weight in kg divided by height in meters squared.

Outcomes

As the patients were DN, we considered homeostasis model of assessment-estimated insulin resistance (HOMA-IR) as the primary outcome measurement. The secondary outcome measurements were FPG, estimated β-cell function (HOMA-B), quantitative insulin sensitivity check index (QUICKI), lipid profiles, biomarkers of inflammation, and oxidative stress.

Assessment of Outcomes

Kidney function was estimated by the Cockroft-Gault formula in mL/min.[15] At baseline and after the 12-week intervention, 10-mL blood samples in a fasting status were obtained from each patient at the university's reference laboratory. Fasting plasma glucose and lipid profiles were quantified on the day blood was collected. Enzymatic kits (Pars Azmun, Tehran, Iran) were used to quantify FPG; serum levels of triglycerides, total cholesterol, very low-density lipoprotein cholesterol (VLDLC), LDLC, high-density lipoprotein cholesterol (HDLC), and creatinine (Jaffe method); and blood urea nitrogen concentrations. Serum insulin concentrations were determined by the use of an enzyme-linked immunosorbent assay kit (Monobind, California, USA). To determine the HOMA-IR, HOMA-B, and QUICKI, we used the suggested formulas.[16] Serum high-sensitivity CRP (HSCRP) concentrations were determined by an enzyme-linked immunosorbent assay kit (LDN, Nordhorn, Germany). Also, we evaluated the plasma nitric oxide by the use of Griess method,[17] total antioxidant capacity using ferric reducing antioxidant power developed by Benzie and Strain,[18] glutathione by the method of Beutler and colleagues,[19] and malondialdehyde concentrations by the method of the thiobarbituric acid reactive substances spectrophotometric test.[20]

Sample Size

To calculate sample size, we used the standard formula suggested for clinical trials by considering type 1 error (α) of 0.05 and type 2 error (β) of 0.20 (power = 80%). Based on a previous study,[8] we used 1.1 as the standard deviation and 0.9 as the difference in mean of HOMA-IR as a primary outcome. Based on this, we needed 25 persons in each group. Assuming 5 dropouts in each group, the final sample size was determined to be 30 individuals per group.

Randomization

Random assignment was done using computer-generated random numbers. Randomization and allocation were concealed from the researchers and participants until the final analyses were completed. The randomized allocation sequence, enrolling participants, and allocating them to interventions were conducted by trained staff at the clinic.

Statistical Analyses

To evaluate whether the study variables were normally distributed or not, we applied the Kolmogrov-Smirnov test. Analyses were conducted based on an intention-to-treat principle. Missing values were treated based on last-observation-carried-forward method,[21] which ignores whether the participant's condition was improving or deteriorating at the time of dropout, but instead freezes outcomes at the value observed before dropout (ie, last observation).[21] To detect differences in anthropometric measures as well as in macro- and micro-nutrient intakes between the two groups, we applied the Student *t* test to independent samples. The chi-square test was used to compare qualitative variables between the two groups. To determine the effects of mulberry extract administration on metabolic profiles, biomarkers of inflammation and oxidative stress, we used the 1-way repeated measures analysis of variance. To identify within-group differences, we applied the paired-samples *t* test. Adjustment for changes in baseline values of biochemical parameters, age, and body mass

index at baseline was performed by the analysis of covariance, using general linear models. *P* values less than .05 were considered significant. All statistical analyses were performed using SPSS software (Statistical Package for the Social Sciences, version 18.0, SPSS Inc, Chicago, IL, USA).

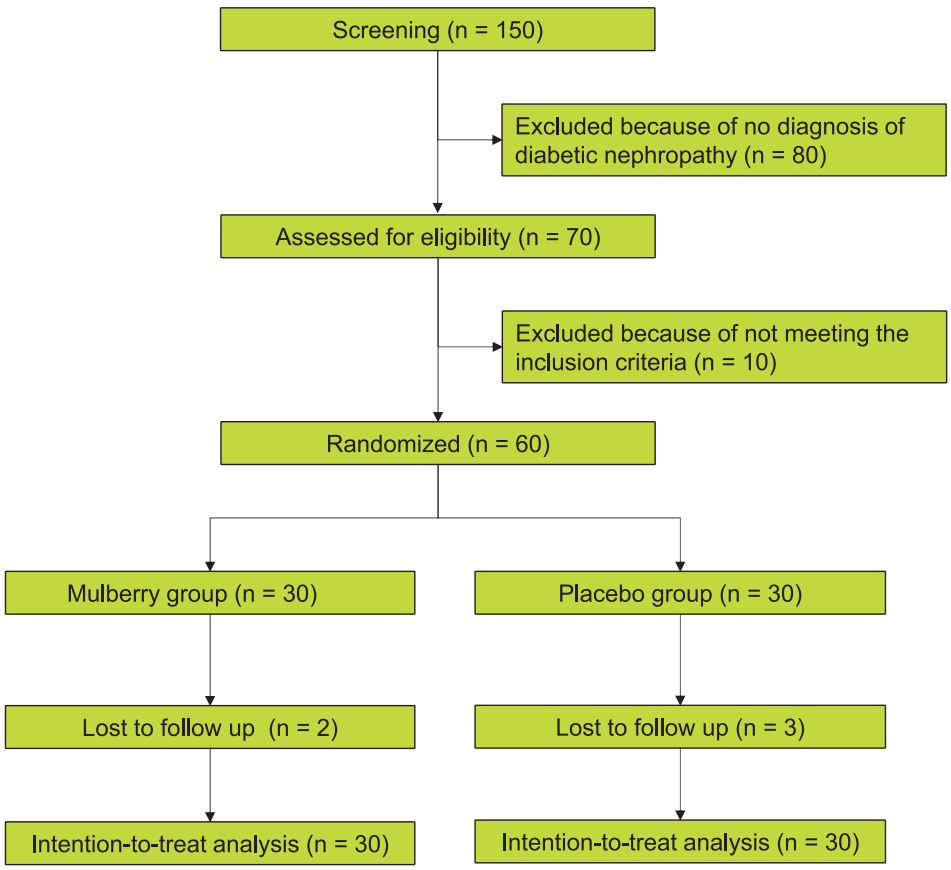
RESULTS

Of 150 diabetic patients who were screened, 80 were excluded from the first visit. Two patients from the mulberry extract group and 3 from the placebo group withdrew due to personal reasons (Figure). Finally, 55 participants (28 in the mulberry extract and 27 in the placebo group) completed the trial. However, we did the analysis based on the intention-to-treat principle; all the 60 participants (30 patients in each group) were included in the final analysis.

Distribution of sex and the mean age, weight, and body mass index at baseline and end of the trial, and weight and body mass index changes of the participants were not different between the two groups (Table 1). In addition, the mean amount of smoking, duration of diabetes mellitus, consumption of antidiabetic and antilipidemia medications, hypertension rate, consumption of angiotensin-converting enzymes inhibitors and angiotensin receptor blockers medications of study participants were not significantly different between the two groups.

Based on the 3-day dietary records obtained at the study baseline, at end of the trial, and throughout the trial (weeks 3, 6, and 9 of the intervention), we found no significant changes in dietary macro- and micro-nutrient intakes between the two groups (data not shown).

After 12 weeks of intervention, compared with the placebo, taking mulberry extract resulted in significant reductions in serum triglycerides (-37.3 ± 64.7 mg/dL versus 3.0 ± 78.8 mg/dL, *P* = .03) and VLDLC (-7.4 ± 12.9 mg/dL versus 0.6 ± 15.8 mg/dL, *P* = .03), and a significant increase in HDLC concentration (0.5 ± 4.0 mg/dL versus -2.0 ± 5.0 mg/dL, *P* = .03; Table 2). In addition,



Summary of patient flow diagram.

Table 1. Characteristics of Studied Participants*

Characteristic	Mulberry Extract Group (n = 30)	Placebo group (n = 30)	*P*
Sex			
Male	7 (23.3)	7 (23.3)	
Female	23 (66.7)	23 (66.7)	> .99
Age, y	63.7 ± 10.8	63.1 ± 9.6	.83
Height, cm	164.0 ± 11.7	160.6 ± 9.1	.22
Weight at the study baseline, kg	83.0 ± 12.2	79.9 ± 9.0	.26
Weight at the end of trial, kg	83.1 ± 12.0	79.9 ± 9.0	.24
Weight change, kg	0.1 ± 0.4	0.01 ± 0.7	.37
Body mass index at the study baseline, kg/m^2	30.9 ± 3.3	31.1 ± 3.9	.81
Body mass index at the end of trial, kg/m^2	30.9 ± 3.3	31.1 ± 3.8	.86
Body mass index change, kg/m^2	0.06 ± 0.2	0.0003 ± 0.3	.56
Smoking	3 (10.0)	3 (10.0)	> .99
Type of diabetes			
Type 1	4 (13.3)	4 (13.3)	
Type 2	26 (86.7)	26 (86.7)	> .99
Duration of diabetes, y	16.1 ± 3.2	15.7 ± 2.9	.58
Insulin therapy	24 (80.0)	23 (76.7)	.75
Antidiabetic medication			
Monotherpy	4 (13.3)	4 (13.3)	
Combination therapy	26 (86.7)	26 (86.7)	.86
Antilipidemic medication			
Monotherpy	16 (72.7)	18 (81.8)	
Combination therapy	6 (27.3)	4 (18.2)	.47
Hypertension	28 (93.3)	28 (93.3)	> .99
Angiotensin-converting enzyme inhibitors	28 (93.3)	28 (93.3)	> .99
Angiotension receptor blockers	5 (16.7)	4 (13.3)	.78

*Values are mean ± standard deviation or frequency (percentage).

changes in serum HSCRP (-2.3 ± 4.5 µg/mL versus -0.1 ± 2.2 µg/mL, $P = .02$), plasma glutathione (+87.8 ± 159.7 µmol/L versus -24.2 ± 138.8 µmol/L, $P = .005$) and malondialdehyde (-0.03 ± 0.5 µmol/L versus 0.7 ± 1.0 µmol/L, $P < .001$) in the supplemented group were significantly different from the changes in these indicators in the placebo group. Supplementation with mulberry extract had no significant effects on markers of insulin metabolism and other lipid concentrations, biomarkers of inflammation, or oxidative stress, compared with the placebo.

Adjustments for baseline values of biochemical parameters, age, and baseline body mass index did not affect our findings (data not shown).

DISCUSSION

In the current study, we evaluated the effects of mulberry extract supplementation on insulin metabolism parameters, lipid profiles, and biomarkers of inflammation and oxidative stress among patients with DN. We found that mulberry extract administration for 12 weeks among DN patients had favorable effects on serum triglycerides, VLDLC, HDLC, HSCRP, glutathione, and malondialdehyde levels; however, it did not affect markers of insulin metabolism, other lipid concentrations, biomarkers of inflammation, or oxidative stress. To our knowledge, this trial is the first indicating the effects of mulberry extract administration on markers of insulin resistance, lipid concentrations, biomarkers of inflammation, and oxidative stress in patients with DN. It must be kept in mind that in the current study, the patients had normal creatinine levels, but proteinuria (> 0.3 g/24 hours) or structural abnormalities or genetic trait point to kidney disease were present.[22] Therefore, GFR may change a bit from one measurement to the next. In some patients, these changes may seem large and enough to move one from one stage to another and then back again.[22] As long as things are not getting progressively worse, it is the average that is important.

Diabetic nephropathy is associated with several

Table 2. Metabolic Profiles, Biomarkers of Inflammation and Oxidative Stress at the Study Baseline and after 3-Month Intervention in Patients With Diabetic Nephropathy That Received Either Mulberry Extract Supplements or Placebo*

Parameter	Mulberry Extract Group				Placebo Group				*P*†
	Baseline	End of Trial	Change	*P*	Baseline	End of Trial	Change	*P*	
FPG, mg/dL	137.6 ± 59.1	113.9 ± 40.8	-23.7 ± 59.2	.03	130.4 ± 33.6	142.6 ± 92.1	12.2 ± 86.6	.44	.06
Insulin, μIU/mL	14.1 ± 6.6	14.5 ± 6.0	0.4 ± 3.7	.48	15.8 ± 7.3	16.1 ± 11.6	0.3 ± 5.9	.77	.89
HOMA-IR	4.7 ± 2.9	4.9 ± 3.0	0.2 ± 1.1	.31	5.1 ± 2.6	5.1 ± 3.5	0.001 ± 1.7	.99	.58
HOMA-B	39.2 ± 25.5	40.5 ± 24.1	1.3 ± 12.6	.59	42.8 ± 24.1	44.7 ± 40.7	1.9 ± 21.3	.62	.88
QUICKI	0.31 ± 0.02	0.31 ± 0.02	-0.003 ± 0.01	.34	0.30 ± 0.02	0.30 ± 0.02	0.002 ± 0.01	.17	.14
Triglycerides, mg/dL	179.8 ± 101.8	142.5 ± 87.3	-37.3 ± 64.7	.004	188.5 ± 101.1	191.5 ± 83.3	3.0 ± 78.8	.83	.03
VLDLC, mg/dL	36.0 ± 20.3	28.5 ± 17.5	-7.5 ± 12.9	.004	37.7 ± 20.2	38.3 ± 16.7	0.6 ± 15.8	.83	.03
Total cholesterol , mg/dL	161.9 ± 36.3	158.7 ± 40.4	-3.2 ± 33.8	.60	159.7 ± 27.0	158.8 ± 29.7	-0.9 ± 12.6	.71	.72
LDLC, mg/dL	89.1 ± 26.9	92.8 ± 27.9	3.7 ± 28.8	.48	78.0 ± 31.8	78.6 ± 30.7	0.5 ± 16.5	.85	.60
HDLC, mg/dL	36.8 ± 7.2	37.3 ± 7.7	0.5 ± 4.0	.49	44.0 ± 7.3	42.0 ± 6.0	-2.0 ± 5.0	.03	.03
HSCRP, μg/mL	7.0 ± 4.7	4.7 ± 4.5	-2.3 ± 4.5	.008	6.5 ± 5.3	6.4 ± 5.4	-0.1 ± 2.2	.75	.02
Nitric oxide, μmol/L	45.4 ± 11.7	50.7 ± 10.3	5.3 ± 5.0	< .001	55.6 ± 11.5	59.2 ± 13.5	3.6 ± 10.6	.07	.42
TAC, mmol/L	1082.4 ± 151.4	1114.6 ± 114.8	32.2 ± 100.1	.08	1180.4 ± 280.7	1177.9 ± 358.3	-2.5 ± 376.0	.97	.62
Total glutathione, μmol/L	662.2 ± 149.4	750.0 ± 112.8	87.8 ± 159.7	.005	626.8 ± 124.4	602.6 ± 146.1	-24.2 ± 138.8	.34	.005
Malondialdehyde, μmol/L	2.1 ± 0.5	2.1 ± 0.6	-0.03 ± 0.5	.75	2.4 ± 0.6	3.1 ± 1.0	0.7 ± 1.0	< .001	< .001
Creatinine, mg/dL	1.9 ± 1.1	2.0 ± 1.2	0.1 ± 1.1	.41	1.5 ± 0.8	1.6 ± 0.8	0.1 ± 0.2	.01	.70
Blood urea nitrogen, mg/dL	30.0 ± 18.7	31.1 ± 19.7	1.1 ± 11.3	.61	27.7 ± 20.1	28.4 ± 21.7	0.7 ± 7.9	.66	.86
GFR, ml/min	62.2 ± 32.4	58.2 ± 32.0	-4.0 ± 19.9	.27	69.5 ± 30.8	66.0 ± 29.1	-3.5 ± 7.2	.01	.89

*FPG indicates fasting plasma glucose; HOMA-IR, homeostasis model of assessment-estimated insulin resistance; HOMA-B, homeostasis model of assessment-estimated B cell function; QUICKI, quantitative insulin sensitivity check index; VLDL, very low-density lipoprotein cholesterol; LDLC, low-density lipoprotein cholesterol; HDLC, high-density lipoprotein cholesterol; HSCRP, high-sensitivity C-reactive protein; TAC, total antioxidant capacity; and GFR, glomerular filtration rate.
†*P* values represent the time × group interaction (computed by the 1-way repeated measures analysis of variance).

metabolic disorders such as insulin resistance, increased biomarkers of inflammation, and oxidative stress.[23-25] Aramwit and coworkers[10] observed that 280 mg of mulberry leaf intake, 3 times per day, resulted in a significant decrease in serum triglycerides and LDLC concentrations among patients with mild dyslipidemia. Furthermore, a significant decrease in total cholesterol, LDLC, and triglycerides, and a significant increase in HDLC levels was seen following the administration of 280 mg of mulberry leaf tablets.[26] In addition, in line with our findings, a 6-month supplementation of mulberry, Korean red ginseng, and banaba did not affect parameters of glucose homeostasis in patients with impaired glucose tolerance and type 2 diabetes mellitus.[27] Long-term ingestion of mulberry leaf extract with enriched 1-deoxynojirimycin content could not influence FPG or glycated hemoglobin concentrations in individuals with impaired glucose metabolism for 12 weeks.[11] The high levels of triglycerides, total cholesterol and LDLC increase the risk of cardiovascular disease, while HDLC has the opposite effect.[28] Therefore, improvement in lipid profiles has been suggested as an effective means to delay vascular complications.

The mechanism of the effect of mulberry leaf lipid concentrations is presently unclear. Due to mulberry ability to reduce serum levels of triglycerides, which is similar to the function of peroxisome proliferator-activated receptor γ (PPAR-γ), it was hypothesized that the function of mulberry administration may be related to the regulation of hepatic expression of PPAR-γ target genes, which in turn are involved in lipid and lipoprotein metabolism.[29] Different findings of the current study with others in terms effect on markers of insulin metabolism and lipid profiles might be mediated by different study designs, the differences in the population studied, dosage of used mulberry as well as period of the study.

The current study demonstrated that mulberry extract intake for 12 weeks among patients with DN had potential to decrease serum HSCRP and plasma malondialdehyde, and to increase plasma glutathione levels compared with the placebo, but unchanged other biomarkers of inflammation and oxidative stress. In agreement with our study, taking 280 mg of mulberry leaf powder 3 times a day demonstrated antioxidant activity as well as decreased CRP levels among mild dyslipidemia patients.[10] Moreover, in another study, it was seen that mulberry had strong immunomodulatory effects on splenocytes, via decreasing the ratios of T helper1 to T helper 2 and pro-to-anti-inflammatory cytokine secretion.[30] In two studies with animal models, mulberry powder intake indicated strong antioxidant property.[31,32] Increased CRP levels in blood can reflect the process involved with inflammation and it is a strong independent predictor of cardiovascular, myocardial infarction, and stroke among healthy individuals.[33] Furthermore, oxidative damage caused by the superoxide and hydroxyl radicals to lipids, proteins, and nucleic acids may trigger various diseases, including cardiovascular disease.[34] Previous studies have demonstrated that the supplementation of antioxidants may decrease the probability of cardiovascular disease.[34] Mulberry intake may produce anti-inflammatory effects through activating PPAR-α and PPAR-γ, inhibit the activation of nuclear factor kappa-light-chain-enhancer of activated B cells, and increase expression of anti-inflammatory cytokines.[35] Flavonols in mulberry including rutin, morin, quercetin, and myricetin are also reported to be effective antioxidants.[36]

The present study had few limitations. The main limitation of the current study was small study numbers. Further long-term studies with higher dose of mulberry extract and sample size are needed to examine the effect of mulberry extract supplementation on markers of insulin resistance, lipid profiles, biomarkers of inflammation, and oxidative stress in DN patients. Another limitation was that we could not assess gene expression involved in the insulin and inflammation signaling pathway in DN patients.

CONCLUSIONS

Overall, our findings showed that mulberry extract administration for 12 weeks had favorable effects on serum triglycerides, VLDLC, HDLC, HSCRP, glutathione, and malondialdehyde levels in DN patients; however, it did not affect markers of insulin metabolism, other lipid concentrations, or biomarkers of inflammation and oxidative stress.

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CONFLICT OF INTERESTS

No conflicted.

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