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Effect of *Cucurbita ficifolia* and Probiotic Yogurt Consumption on Blood Glucose, Lipid Profile, and Inflammatory Marker in Type 2 Diabetes

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ABSTRACT

Background: Control of blood sugar, hypertension, and dyslipidemia are key factors in diabetes management. *Cucurbita ficifolia* (pumpkin) is a vegetable which has been used traditionally as a remedy for diabetes in Iran. In addition, consumption of probiotics may have beneficial effects on people with Type 2 diabetes. The aim of this study was an investigation of the effects of *C. ficifolia* and probiotic yogurt consumption alone or at the same time on blood glucose and serum lipids in diabetic patients.

Methods: Eighty eligible participants randomly were assigned to four groups: 1 - green *C. ficifolia* (100 g); 2 - probiotic yogurt (150 g); 3 - *C. ficifolia* plus probiotic yogurt (100 g *C. ficifolia* plus 150 g yogurt); and 4 -control (dietary advice) for 8 weeks. Blood pressure, glycemic response, lipid profile, and high-sensitive C-reactive protein (hsCRP) were measured before and after the intervention.

Results: Total cholesterol (TC) decreased significantly in yogurt and yogurt plus *C. ficifolia* groups (within groups $P = 0.010$, and $P < 0.001$, respectively). *C. ficifolia* plus yogurt consumption resulted in a decrease in triglyceride (TG) and an increase in high-density lipoprotein cholesterol (HDL-C) (within groups $P < 0.001$ and $P = 0.001$, respectively). All interventions led to a significant decrease in blood sugar, hemoglobin A1c (HbA1c), hsCRP, and low-density lipoprotein cholesterol (LDL-C) level within groups. Blood pressure decreased significantly in Cucurbita group and yogurt group (within groups $P < 0.001$, and $P = 0.001$ for systolic blood pressure [SBP] and $P < 0.001$, and $P = 0.004$ for diastolic blood pressure [DBP], respectively). All variables changed between groups significantly except LDL-C level.

Conclusions: Variables including TG, HDL-C, TC, fasting blood sugar, HbA1c, SBP, DBP, and hsCRP changed beneficially between groups. It seems that consumption of *C. ficifolia* and probiotic yogurt may help treatment of diabetic patients.

Keywords: Glycemic response, inflammatory marker, lipid profile, probiotic yogurt, pumpkin, Type 2 diabetes

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INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a chronic disease caused by insulin resistance and consequent decline in peripheral glucose uptake. Sedentary lifestyle, obesity, and unhealthy dietary behaviors are the most common risk factors of T2DM.[1] It was estimated that diabetes prevalence was 4% in 2010 and was expected to reach 5.4% by 2025.[2] The burden of T2DM is predicted to double in the near future. Approximately, 7.7% or 2 million adults suffer from diabetes in Iran. Diabetes prevalence has been reported 7% and 8% in Isfahan and Tehran provinces, respectively.[3] T2DM leads to serious complications such as nervous system disorders, kidney diseases, and eye problems, thus its prevention and treatment should be considered an urgent priority.[4] Medicinal plants have been used in the treatment of this disease as a supplementary method. There are more than 800 plants that have been utilized as an experimental treatment for DM. Their active compounds that have hypoglycemic effects included mucilage gum, glycans, flavonoids, triterpenes, and alkaloids. One of these plants that has been used traditionally in Asia is *Cucurbita ficifolia* (*Cucurbitaceae*) popularly known as pumpkin.[5,6] Initial investigations indicated that *C. ficifolia* may reduce blood glucose and lipid profile and improve glucose tolerance.[7] However, the mechanism of anti-diabetic action of this vegetable is unknown. Probiotic foods have live organisms which are beneficial for health. There are some evidences that probiotic foods consumption or supplementation might reduce serum cholesterol level and improve insulin sensitivity.[8] Some studies have evaluated the favorable health effects of probiotic dairy products. They showed that probiotic yogurt had a beneficial effect on metabolic factors and health cardiovascular health parameters.[9-12]

Since, there is little information regarding the effects of *C. ficifolia* and probiotic foods on blood glucose levels in humans, the goal of this study was to determine the effects of *C. ficifolia* and/or probiotic yogurt consumption on glycemic control, lipid profile, and inflammatory markers in Type 2 diabetic patients.

METHODS

Study design and participants

We conducted a parallel-group randomized controlled clinical trial. Type 2 diabetic patients were recruited from the Endocrinology and Metabolism Research Center, Isfahan. Eligibility criteria were age between 25 and 75 years, fasting blood sugar (FBS) more than 126 mg/dL, and controlled blood lipid without changing the drug instruction. All participants had to be no-smokers, take metformin or glibenclamide to control blood sugar, and not to drink any kinds of alcoholic beverages. Patients were excluded if they had a history of chronic illnesses such as renal, liver, pulmonary, and heart diseases or pancreatitis, endocarditis, short bowel syndrome, and allergy. None of the participants had autoimmune disorders were pregnant or lactating mothers. Totally, 80 Type 2 diabetic patients were selected and asked to participate in the study. The study protocol was approved by the Ethics Committee at Isfahan University of Medical Sciences, and all participants completed an informed consent form. Flow chart of study participants was shown in Figure 1.

Procedures

After enrollment, the subjects were randomly assigned to one of four dietary intervention arms lasting 8 weeks. During the intervention, subjects took one of following diets: (1) *C. ficifolia* (100 g); (2) probiotic yogurt (150 g); (3) *C. ficifolia* and probiotic yogurt (100 g *C. ficifolia* plus 150 g yogurt); and (4) control (dietary advice). Patients were instructed on consuming *C. ficifolia* and yogurt at lunch. They were required to consume *C. ficifolia*. Subjects were asked not to change their dietary habits, physical activity level, or other lifestyle factors during the study. Dietary compliance was assessed by regular weekly contacts or text messages. We also took a 3-day diet recall to assure compliance and analyzed it with Nutrition-IV software, version 15 (First Databank, San Bruno, CA, USA). This clinical trial was registered at irct.ir with number IRCT2013041311763N7.

Laboratory analyses

Five milliliter of fasting blood samples were obtained at baseline and at the end of the study. Plasma and serum stored at −70°C for determination of glucose, lipids, and CRP. Total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and triacylglycerols were measured by autoanalyzer. The Friedewald equation was used to calculate low-density lipoprotein cholesterol (LDL-C),[13] when plasma triglyceride (TG) concentration was <400 mg/dL. Blood glucose (glucose oxidase method)[14] and hemoglobin A1c (HbA1c) were determined by an autoanalyzer. Serum concentrations of high sensitive C-reactive protein (hsCRP) were determined by immune turbidimetric using PARS AZMOON kit.

Statistical analysis

All quantitative variables have been reported as mean ± standard deviation, and qualitative variables as frequency (percent). Normality of variables was evaluated using K-S test or P-P plot. For nonnormal variables, log transformation was used. One-way analysis of variance (ANOVA) and Chi-square were used to assess baseline differences among the groups. Paired *t*-test was used to evaluate the difference between baseline and final values. Further analyses were

conducted to investigate the between-group comparisons using multivariate ANOVA or multivariate analysis of covariance as appropriate. Bonferroni *post hoc* test was used for pairwise comparisons. The value $P < 0.05$ was considered as statistically significant level. Analyses were performed using SPSS 15.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

All 80 subjects completed the study (28.4 ± 2.9 y; body mass index, 23.1 ± 0.9 kg/m^2). The characteristics of the patients showed no significant differences between groups [Table 1]. There was no significant difference between groups in total energy intake, macronutrient intake, and body weight at baseline. At the end of the study, no statistically significant differences between groups were observed for dietary intakes [Table 2]. Baseline values of biochemical measures were not different between groups.

After 8 weeks, there were no significant changes in TC and LDL-C in the control group, but TG increased ($P = 0.012$), and HDL-C decreased ($P = 0.034$) significantly. In all intervention groups TC decreased, but it was significant only in yogurt and yogurt plus *C. ficifolia* groups ($P = 0.010$, and $P < 0.001$, respectively). TG declined, and HDL-C enhanced significantly in *C. ficifolia* plus yogurt group ($P < 0.001$and $P = 0.001$, respectively). Yogurt consumption decreased LDL-C significantly ($P = 0.003$ in yogurt and $P = 0.004$ in *C. ficifolia* plus yogurt). All changes were significant between groups ($P < 0.001$) except for LDL-C.

All interventions significantly decreased FBS ($P = 0.001$ in pumpkin, $P = 0.014$ in yogurt, and $P < 0.001$in *C. ficifolia* plus yogurt) and HbA1c ($P < 0.001$in *C. ficifolia*, $P = 0.002$ in yogurt, and $P = 0.000$ in *C. ficifolia* plus yogurt) in comparison with control group (between group *P*, 0.001 and < 0.001, respectively). HsCRP showed a significant reduction in all intervention groups ($P < 0.001$) which was more significant in *C. ficifolia* and *C. ficifolia* plus yogurt groups compared with yogurt group. At the end of the study, a statistically significant difference was seen among groups in systolic blood pressure (SBP) and diastolic blood pressure (DBP). Blood pressure decreased in *C. ficifolia* group and yogurt group significantly ($P < 0.001$, and $P = 0.001$ for SBP and $P < 0.001$, and $P = 0.004$ for DBP, respectively) [Table 3].

Table 1: Baseline characteristics of participants in all groups

Variables	Control	Green pumpkin	Yogurt	Green pumpkin plus yogurt	*P*
Age (year)	46.95±9.34	51.8±2.24	54.1±9.54	53.65±6.99	0.08
Sex (% male)	45	60	15	20	0.09
Height (cm)	163.05±10.36	163±10.21	158.8±7.26	160.2±7.99	0.36
Weight (kg)	79.23±14.81	76.83±10.73	72.5±12.09	71.67±9.77	0.15
BMI (kg/m^2)	29.75±4.66	28.95±3.34	28.77±4.59	27.98±4.2	0.62

BMI=Body mass index

Table 2: Comparison of nutrients intake in all groups of study

Variables	Control	Green pumpkin	Yogurt	Green pumpkin plus yogurt	*P*
Energy (kcal)	2059.71±494.08	2232.09±638.52	2069.53±506.12	2213.04±647.77	0.556
Carbohydrate (g)	253.87±28.69	320.68±27.78	302.63±75.03	346.92±150.67	0.131
Protein (g)	51.16±72.72	63.47±49.45	67.52±20.64	69.18±20.06	0.954
Total fat (g)	60.56±23.25	40.81±26.13	69.61±29.43	62.66±27.96	0.972
SFA (g)	10.00±5.47	10.77±3.63	11.50±5.89	12.59±5.63	0.580
MUFA (g)	13.81±14/48	11.69±6.95	11.12±4.29	15.09±7.63	0.610
PUFA (g)	13.73±10.24	13.09±11.12	10.24±4.34	13.61±5.49	0.630
Vitamin C (mg)	87.72±66.60	54.00±47.16	92.21±63.28	75.03±30.62	0.221
Vitamin D (pg)	0.30±0.54	0.25±0.63	0.56±1.04	0.62±0.94	0.507
Sodium (mg)	2038.1±878.64	2563.0±1221.48	2718.1±1819.71	2384.6±1120.14	0.527
Potassium (mg)	734.37±2662.2	2437.0±649.08	573.57±2580.9	489.37±2654.9	0.733
Calcium (mg)	790.09±230.34	798.41±336.93	772.49±355.00	832.63±357.19	0.960
Total fiber (g)	3.20±6.48	6.24±2.70	6.66±3.53	6.65±2.43	0.978
Soluble fiber (g)	0.539±0.261	0.299±0.256	0.520±0.289	0.462±0.330	0.101
Insoluble fiber (g)	3.97±3.00	2.20±1.25	2.92±1.28	3.42±2.35	0.138
Sucrose (g)	11.66±9.10	7.18±6.42	10.23±6.12	10.09±6.32	0.378
Glucose (g)	8.44±5.59	8.19±2.92	8.96±3.90	8.15±3.78	0.947

SFA=Saturated fatty acid, MUFA=Monounsaturated fatty acid, PUFA=Polyunsaturated fatty acid

Table 3: Blood pressure, fasting blood glucose, hemoglobin A1c, serum lipids, and high sensitive C-reactive protein at baseline and postintervention in the four groups

Variables	Control	Green pumpkin	Yogurt	Green pumpkin plus yogurt	P¥
SBP (mmHg)					
Before	127.0±13.41	125.0±17.01	124.50±12.76	125.0±14.68	0.004
After	125.50±14.68	113.50±11.82	117.50±9.10	122.0±11.05	
P*	0.48	<0.001	0.001	0.23	
DBP (mmHg)					
Before	85.50±9.44	86.0±9.94	83.0±8.01	82.50±10.69	0.001
After	85.0±10.51	77.50±7.16	78.50±5.87	81.0±9.67	
P*	0.71	<0.001	0.004	0.37	
FBS (mg/dL)					
Before	145.20±41.90	156.10±43.35	148.95±47.26	180.80±68.68	0.001
After	165.50±41.34	123.95±21.98	126.25±34.01	115.0±22.40	
P*	0.02	0.001	0.01	<0.001	
HbA1c (%)					
Before	7.54±2.03	7.21±0.91	7.06±1.58	7.97±1.265	<0.001
After	7.55±1.87	6.41±0.78	6.49±1.33	6.40±1.18	
P*	0.98	<0.001	0.002	<0.001	
TC (mg/dL)					
Before	178.9±54.97	188.80±32.53	188.85±49.22	189.0±43.05	<0.001
After	189.35±46.36	168.70±35.79	165.7±42.37	150.15±40.78	
P*	0.29	0.053	0.01	<0.001	
TG (mg/dL)					
Before	165.20±65.58	204.05±148.23	190.65±129.11	203.70±87.50	<0.001
After	206.80±93.24	146.30±48.37	142.70±55.0	127.15±37.31	
P*	0.01	0.07	0.06	<0.001	
LDL-C (mg/dL)					
Before	110.66±41.68	121.14±28.62	107.63±34.97	109.03±40.43	0.150
After	106.55±29.19	95.41±28.63	93.82±27.80	92.03±31.60	
P*	0.56	0.003	0.057	0.004	
HDL-C (mg/dL)					
Before	44.45±9.43	37.70±7.48	44.80±10.04	40.55±9.09	<0.001
After	40.34±11.50	39.70±9.17	48.40±10.49	51.50±12.71	
P*	0.03	0.30	0.07	0.001	
hsCRP					
Before	1.16±0.24	1.40±0.18	1.29±0.27	1.69±0.25	<0.001
After	1.24±0.32	1.16±0.20	1.13±0.29	1.13±0.34	
P*	0.14	<0.001	0.006	<0.001	

*Within group comparisons based on paired *t*-test, ¥Between group comparisons based on MANOVA. DBP=Diastolic blood pressure, SBP=Systolic blood pressure, FBS=Fasting blood sugar, TC=Total cholesterol, TG=Triglyceride, HDL-C=High-density lipoprotein cholesterol, LDL-C=Low-density lipoprotein cholesterol, MANOVA=Multivariate analysis of variance, HbA1c=Hemoglobin A1c, hsCRP=High sensitive C-reactive protein

DISCUSSION

In patients with Type 2 diabetes, it appears that probiotic yogurt consumption may substantially influence TC, FBS, HbA1c, hsCRP, and blood pressure. *C. ficifolia* significantly decreased LDL-C, glycemic control, hsCRP, and blood pressure. The most favorable effects on lipid profile, FBS, HbA1c, and hsCRP were related to *C. ficifolia* plus probiotic yogurt.

A few studies on human have shown that probiotic foods may decline serum lipids and lipoproteins.[15-17] The only study which used probiotic yogurt showed that TC decreased in mild to moderate hyperlipidemic subjects, but the other components of lipid profile did not change.[16] In this study, *C. ficifolia* just decreased LDL-C but animal studies revealed that it may reduce TG and increase HDL-C too.[18-20] The most noticeable results were related to *C. ficifolia* plus yogurt, which decreased atherogenic lipids and lipoproteins and increased HDL-C. To the best of our knowledge, no study has evaluated the combination of these two foods. The amount of pectin in *C. ficifolia* is high which can increase bile salts excretion

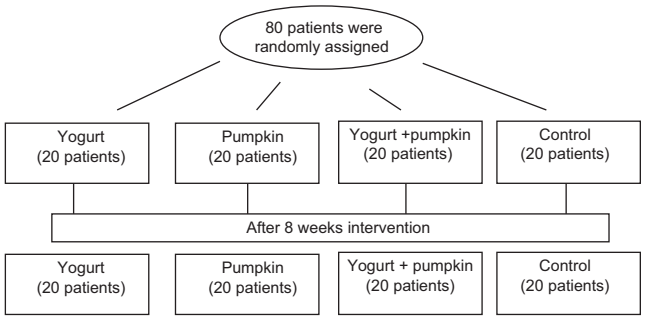

Figure 1: Flow chart of study participants

and lipoprotein lipase activity and consequently decrease serum lipid levels.[19] The mechanism by which probiotic yogurt cholesterol reduces cholesterol can be attributed to the removal of the cholesterol by assimilation, its incorporation into the cell membrane, deconjugating bile acids,[21] and inhibition of hydroxy-methyl-glutaryl-CoA reductase (HMG-CoA reductase).[22]

The results of the study in the subjects with Type 2 diabetes indicated that yogurt and *C. ficifolia* alone or in combination decreased FBS and HbA1c significantly. Previous studies in diabetic subjects and also rats have shown that blood glucose and insulin sensitivity improved after probiotic consumption.[23] This finding contrasts the report by Mazloom *et al.* which concluded the relationship between probiotic capsule intake and FBS and insulin level.[15] This study did not assess participants' compliance that can confound the results. The positive effects of *C. ficifolia* on glycemic control, insulin sensitivity, and glucose tolerance were reported by other researchers in animal models.[15,24-28] This finding can be explained by *C. ficifolia* effects in terms of β-glucosidase α-amylase inhibition,[24] and pancreas function and insulin sensitivity improvement.[15,29] The amount of fiber in this vegetable is not enough to produce such a result,[24] but it might be related to D-chiro inositol.[18]

HsCRP decreased in all intervention groups significantly. Moroti *et al.*, evaluated the effects of *C. ficifolia* consumption on diabetic rats and found a reduction in CRP.[23] They assumed that it dropped because of the flavonoid content of *C. ficifolia*. Hadisaputro *et al.* found similar findings with kefir consumption on interleukin (IL)-1 and -6.[30] However, Mazloom *et al.* results were nonsignificant for IL-6 reduction, and they reported an increase in CRP.[15] This inconsistency can be described by the fact that they used probiotic as a supplement in diabetic patients. The role of probiotic in inflammation reduction may possibly be associated with gut microflora modulation.[31,32]

In this study, yogurt and *C. ficifolia* intake relative to their combination reduced SBP and DBP. Agerholm-Larsen *et al.* showed that 8 weeks intake of probiotic milk products decreased SBP significantly in healthy obese and overweight people.[33] Daily ingestion of the probiotic dairy such as fermented milk can reduce blood pressure in the high normal to mild hypertensive subjects.[34] Probiotics produce angiotensin-converting enzyme peptides by microbial activity which can play a role in blood pressure reduction.[35] It has not yet been defined any special mechanism for blood pressure lowering effect of *C. ficifolia*. As oxidative stress can lead to an increase in peripheral vascular resistance and hypertension[36] and Cucurbita decreases lipid peroxidation index such as thiobarbituric acid reactive substances and malondialdehyde and increases glutathione activity,[18,27] this outcome can be partly explained. However, we could find no interpretation for the lack of impact of yogurt and *C. ficifolia* combination on blood pressure.

CONCLUSIONS

Our results suggest that probiotic yogurt and *C. ficifolia* alone or together have beneficial effects on lipid profile, glycemic control, inflammation, and blood pressure in Type 2 diabetic patients. Our data confirm that concomitant use of *C. ficifolia* and yogurt has more noteworthy results in comparison with their intake alone. Variables including TG, HDL-C, TC, FBS, HbA1c, SBP, DBP, and hsCRP changed beneficially between groups. It seems that consumption of *C. ficifolia* and probiotic yogurt may help treatment of diabetic patients. Conducting more research in this area seems necessary to elucidate mechanisms and other effects.

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Conflicts of interest
There are no conflicts of interest.

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