Effects of Hesperidin Supplementation on Glycemic Control, Lipid Profile and Inflammatory Markers in Patients with Type 2 Diabetes: A Randomized Double Blind Placebo Control Clinical Trial

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ABSTRACT

This study was aimed to investigate effects of hesperidin (a common constituent of citrus fruits) supplementation on indices of glycemic control, insulin resistance, lipid profile, and inflammatory markers in patients with type 2 diabetes. Forty-five patients participated in this randomized, double-blind controlled clinical trial. Patients were randomly divided into 2 groups of intervention and control. Participants consumed either 500 mg/d pure hesperidin supplement or placebo in intervention and control groups for 8 weeks, respectively. Hesperidin supplementation led to significant decrease in fasting blood glucose and glycated hemoglobin A1c ($p = 0.041$ and $p = 0.028$, respectively). A significant increase in serum insulin ($p = 0.018$) and a decrease in total cholesterol ($p = 0.049$) were also observed in hesperidin group, whereas no significant changes occurred in placebo group. Inflammatory markers, high-sensitivity C-reactive protein (hs-CRP) and interleukin-6 (IL-6) did not significantly change in hesperidin group compared to the control group. In conclusion, hesperidin supplementation not only lowered plasma level of total cholesterol but also improved glycemic control and insulin levels in patients with type 2 diabetes.

Keywords: Glycemic control, Hesperidin, Inflammatory markers, Insulin resistance, Lipid profile, Type 2 diabetes

INTRODUCTION

Diabetes mellitus is a common chronic disease characterized by high level of blood glucose, insulin resistance and abnormal insulin secretion [1]. Increasing prevalence of diabetes mellitus is considered a global public health concern. According to the World Health Organization, 210 million people were suffering from diabetes in 2010 worldwide [2]. Type 2 diabetes is a much more prevalent form of diabetes and responsible for 90% of the disease prevalence [3]. This type of diabetes is associated with several complications such as obesity, hypertension, and hyperlipidemia

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that may lead to cardiovascular diseases [4]. Inflammation plays an important role in development of type 2 diabetes and its complications [5]. Diabetes can also have a deleterious effect on immune function [6]. Increased levels of pro inflammatory markers including high-sensitivity C-reactive protein (hs-CRP), tumor necrosis factor-alpha (TNF-α), and interleukin-6 (IL-6) trigger an inflammatory cascade, systemic insulin resistance, and β-cell dysfunction in addition to increasing the risk of cardiovascular events [7]. A number of drugs have been used for the treatment of type 2 diabetes. These drugs act either by enhancing insulin secretion or by improving insulin sensitivity and when used alone or in combination together can be effective, but they are frequently constrained by safety, tolerability, hypoglycemia, lactic acidosis, weight gain and GI disturbances [8]. Hence for better safety and potential therapeutic value, the search for novel molecules that offers better protection and fewer side effects is warranted [9].

Diet is known to have a crucial impact on the main risk factors which are responsible for cardiovascular complications in patients with type 2 diabetes. Diet can exert its effects by modulating plasma levels of lipids and lipoproteins, blood pressure, energy balance and oxidative modification or protection of plasma lipids and lipoproteins [4]. Previous epidemiologic studies suggested that high intake of fruits and vegetables are associated with a reduced risk of coronary heart disease [10,11]. These beneficial effects could be related to minor components, especially flavonoids, which are proposed to exert their action by inhibiting low density lipoprotein cholesterol (LDL-C) oxidation and platelet aggregation [12]. Bioflavonoids have been reported to improve hyperglycemia by affecting glucose transport and insulin receptor function [13]. The proposed mechanisms underlying the protective role of flavonoids include regulating postprandial glucose, delaying gastric emptying rate, and reducing active transport of glucose across intestinal brush border membrane [14]. Inhibition of intestine sodium-glucose cotransporter-1 (Na-Glut-1) along with inhibition of α-amylase and α-glycosidase activity makes flavonoids work as potential candidate factors in the management of hyperglycemia [15].

The bioflavonoid, hesperidin, is a specific glycoside which is frequently found in oranges and lemons. Hesperidin exhibits biological and pharmacological properties such as anti-inflammatory, anticarcinogenic, lipid-lowering and antioxidant activities [13]. However, there is limited number of publications assessing the antidiabetic effects of hesperidin [16]. Kurowska et al. reported that daily consumption of 750 ml orange juice can increase high density lipoprotein cholesterol (HDL-C) concentrations by 21% and triglyceride (TG) levels by 30% in subjects with hypercholesterolemia [17]. Although their purported properties suggest that hesperidin could be useful for improvement of diabetes control and prevention of the development of its chronic complications, the possible benefits of hesperidin administration as an adjuvant therapy for the treatment of type 2 diabetes, based on some randomized controlled trials, are limited and deserve further investigation [18]. Therefore, this study was aimed to evaluate the efficacy of hesperidin on impaired glucose tolerance, insulin resistance and some biochemical parameters in order to find out whether results of which could lead to improve the management of patients with diabetes as an adjuvant therapy.

MATERIALS AND METHODS

This study was approved by the Ethics Committee of Iran University of Medical Sciences and it was registered on Iranian Registry of Clinical Trials website (IRCTID: IRCT201407242602N12). Forty-eight participants with type 2 diabetes who met inclusion criteria were included in the study from Endocrine Research Center, Institute of Endocrinology and Metabolism, Iran University of Medical Sciences. Inclusion criteria consisted of having type 2 diabetes at least one year and voluntary consenting for participation in the study. Diabetes was defined according to the recommendations of the American Diabetes Association's Expert Committee on the Classification and Diagnosis of Diabetes [19]. Subjects were excluded if they had hepatic disorders, renal failure, cardiovascular

disease, celiac or gastrointestinal disorders, rheumatoid arthritis, endocrine and thyroid disorders, leukemia and inborn errors in the enzymes that metabolize homocysteine. In addition, subjects who had insulin therapy, nutrient supplementation, high consumption of flavonoid-rich beverages (including green tea and coffee), smoking, alcohol intake and using corticosteroids, anticonvulsants or isoniazid were excluded. All patients gave their written informed consent in order to participate in the study.

After a double-blind manner, each participant was assigned randomly into one of the two groups: In the intervention group, patients (n = 24) received hesperidin supplementation for 8 weeks (500 mg daily), and in the placebo group, patients (n = 24) received the same dose of placebo. The placebo tablets were similar to hesperidin tablets in both appearance and taste. Both hesperidin and placebo supplements were produced by Herbal Extracts Plus Company (Croydan, PA, USA) and the School of Pharmacy, Tehran University of Medical Sciences, respectively. Participants in two groups were matched for age and body mass index (BMI) and they were also advised to avoid changing their habitual diet or exercise levels during the study.

Blood samples were taken after an overnight fasting at the beginning of the study and after two months of supplementation at the end. The blood samples were centrifuged, and their serums were stored at -80°C until they were treated and analyzed further. Glucose and lipid profile [total cholesterol (TC), HDL-C, LDL-C and TG] were analyzed on the day of sampling. The levels of fasting blood glucose (FBG), TC, HDL-C and TG were determined by the enzymatic method and using an Abbot Model Aclyon 300 auto-analyzer with Pars-Azmoon kits (Tehran, Iran). LDL-C levels were calculated by the Friedwald equation [20]. Glycated hemoglobin (HbA1c) was measured by using an automated high performance liquid chromatography analyzer with a kit of Bio-Rad D-10 Laboratories, Schiltigheim, France. Serum insulin concentration was measured by chemiluminescent immunoassay (CLIA) method [LIAISON analyzer (310360) Diasorin S.P.A, Vercelli, Italy]. Homeostasis model assessment-insulin resistance (HOMA-IR) was calculated by the following formula: fasting glucose (mg/dL) * fasting insulin (μU/mL) / 405 [21]. Serum hs-CRP concentration was determined by using an immune turbidimetric assay (Pars Azmoon Co., Tehran, Iran). IL-6 levels were measured with an enzyme-linked immunosorbent assay kit (Diaclon, France).

Participants' systolic and diastolic blood pressure was measured with a standard mercury sphygmomanometer after a 15 minute rest in a seated position. Anthropometric parameters including height and weight were measured at the beginning and end of the study. BMI was calculated by using the recorded data of height and weight. The dietary data were collected by a 24-h dietary recall for three days (one holiday day and two usual days) at the beginning, at the end of the fourth week and at the end of the study. The average daily nutrient intake was calculated by using modified Nutritionist IV software (version 3.5.2, First Data Bank; Hearst Corp, San Bruno, California). Moreover, physical activity for each subject was assessed by using a short form of International Physical Activity Questionnaire (IPAQ) at baseline and at the end of the study [22].

For estimating the sample size, we used this formula: $N = (Z_{1-\alpha/2} + Z_{1-\beta})^2 * (S_1^2 + S_2^2) / (\mu_1 - \mu_2)^2$ where type one (α) and type two errors (β) were 0.05 and 0.20 (power = 80%), respectively. According to a previous study, the variances of hs-CRP in the case and control groups were 2.1 and 1.6, respectively. We also considered 1.6 as the difference in the mean ($\mu_1 - \mu_2$) of hs-CRP [23]. The formula showed that the present study needed 21 participants in each group for 80 % of the study power. Normal Distribution of Data was assessed by Kolmogorov-Smirnov test. Data are reported as means ± standard deviations. Comparisons of baseline and endpoint values between groups were done by independent t test after 2 months of intervention. Paired t test was used for within-group comparisons (pre-and post-intervention values in each group). All statistical analyses were conducted with Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA) version 18. The differences with P-values < 0.05 were considered significant.

RESULTS AND DISCUSSION

Forty-five participants (23 in hesperidin group and 22 in placebo group) of 48 enrolled subjects completed the study. Three patients, 1 in hesperidin group and 2 in placebo group withdrew from the study because of non-compliance with the intervention (Figure 1). The mean age for case and control groups were 53.21±6.29 and 53.40±7.49 years, respectively (P=0.92). Also, there was no significant difference between two groups with regard to sex distribution (P=0.90). No adverse events were reported during the study.

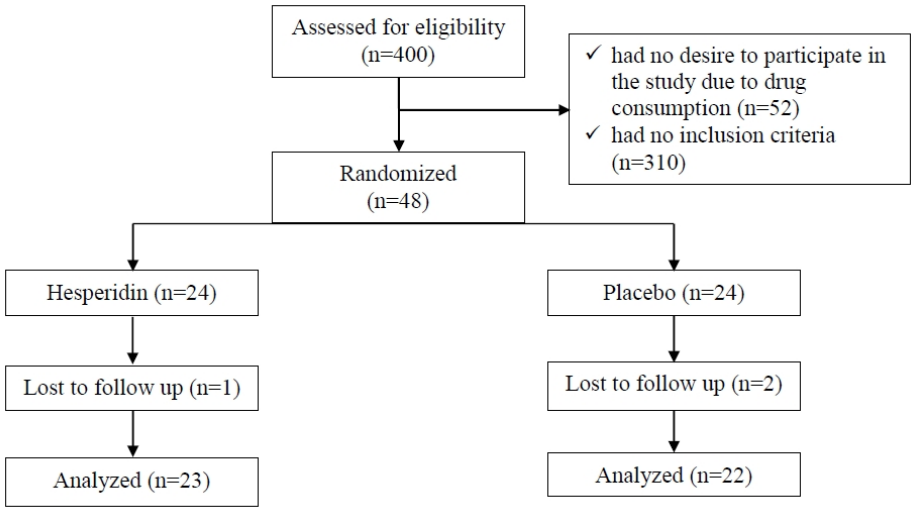

Figure 1. Summary of patient enrollment.

Anthropometric characteristics and blood pressure of subjects have been illustrated in table 1. There were no significant differences at the baseline and end of the study regarding anthropometric indices and blood pressure between the groups. Dietary intake of energy, carbohydrate, protein and fat, determined by a 3-day food recall, were not significantly different between the groups before and after the intervention (Table 2).

Effects of hesperidin on glycemic control, lipid profile and inflammatory markers are presented in table 3. After 8 weeks of supplementation, FBG, HbA1c and insulin levels changed significantly ($P < 0.041$, < 0.028 and < 0.018, respectively) whereas HOMA-IR remained unchanged in both groups. Serum insulin levels increased considerably in hesperidin group after the intervention compared to the beginning of the study.

There were no significant differences in lipid profile in participants of two groups at baseline. There were not any significant changes in lipid profile including TG, LDL-C and HDL-C levels in hesperidin group compared with the placebo group either. The mean of TC declined significantly after 8 weeks of hesperidin supplementation ($P < 0.049$). No Significant differences in baseline levels of hs-CRP and IL-6 were observed. Inflammatory markers were not significantly changed in hesperidin group compared with control group after 8 weeks.

In this clinical trial, we have investigated to our best knowledge for the first time the effect of daily consumption of pure hesperidin on indices of glycemic control, insulin resistance, lipid profile, inflammatory markers, and blood pressure in patients with type 2 diabetes. This study showed that

consumption of hesperidin, one of the major orange flavonoid constituents, at the level of 500 mg/d for eight weeks could lead to significant decreases in serum levels of FBG, HbA1c and TC and increases in serum levels of insulin in type 2 diabetic patients.

Table 1. Anthropometric characteristics and blood pressure of study participants*.

Variables	Group	Before	After	Mean change from base to end	P2
Weight (kg)	Hesperidin	73.58±11.00	73.52±11.71	-0.06±1.63	0.961
	Placebo	73.47±7.49	73.20±7.37	0.27±1.42	0.375
	P1	0.964	0.973	0.653	
BMI (kg/m2)	Hesperidin	26.96±2.58	26.74±2.61	-0.22±0.82	0.331
	Placebo	27.05±3.75	26.87±6.42	-0.18±4.60	0.230
	P1	0.932	0.586	0.336	
DBP (mmHg)	Hesperidin	8.22±0.92	8.17±0.79	-0.05±1.12	0.693
	Placebo	8.43±0.87	8.27±0.37	-0.16±0.91	0.241
	P1	0.385	0.811	0.973	
SBP (mmHg)	Hesperidin	12.21±1.64	12.26±1.75	0.05±1.34	0.724
	Placebo	12.87±1.46	12.36±0.52	-0.51±1.36	0.081
	P1	0.15	0.954	0.175	

*Data were expressed as mean±SD, P1 was resulted from independent sample t test, P2 was resulted from paired sample t test, BMI: Body Mass Index, DBP: Diastolic Blood Pressure, SBP: Systolic Blood Pressure

Table 2. Dietary intakes of participants at baseline and at the end of the study*.

Variables	Group	Before	After	Mean change from base to end	P2
Energy(kcal/day)	Hesperidin	1760.13±273.89	1785±299.76	25±125.02	0.323
	Placebo	1692.21±432.42	1785±299.76	93±134.86	0.566
	P1	0.518	0.760	0.263	
Protein (g/day)	Hesperidin	71.47±14.73	75.69±17.56	4.22±14.36	0.193
	Placebo	80.04±19.23	74.33±16.78	-5.71±15.19	0.1
	P1	0.103	0.794	0.10	
Fat (g/day)	Hesperidin	20.69±20.69	22.21±22.21	1.52±16.28	0.686
	Placebo	58.33±18.16	65±23.88	6.67±20.60	0.154
	P1	0.230	0.775	0.357	
Carbohydrate(g/day)	Hesperidin	221.47±55.14	225.78±50.36	4.31±29.84	0.308
	Placebo	238.33±38.79	227.38±46.58	-10.95±42.12	0.274
	P1	0.252	0.518	0.170	

*Data were expressed as mean±SD, P1 was resulted from independent sample t test, P2 was resulted from paired sample t test

Many studies have shown that some flavonoids and other phytochemicals can decrease blood glucose levels [24]. The results of Jung et al. study also revealed the anti-hyperglycemic effect of hesperidin and naringin in mice [25]. Moreover, there was evidence to support of anti-hyperglycemic effect of naringin in streptozotocin (STZ) / nicotinamide diabetic rats [26]. Another study also demonstrated that hesperidin supplementation potentially ameliorated elevated levels of glucose in type 2 diabetic rats [27]. Alterations in the activity of key enzymes involved in glucose metabolism might be one of the reasons associated with beneficial effects of flavonoids on diabetes. It has been reported that hesperidin increases mRNA level of glucokinase, a key enzyme of glucose catabolism, and decreases level of G6Pase, a gluconeogenic enzyme, in type 2 diabetic mouse [28]. Estimation of HbA1c has been found to be particularly useful in monitoring the effectiveness of

therapy in diabetes [29]. In our study, oral administration of hesperidin significantly decreased the levels of HbA1c. This study revealed a significant increase in fasting insulin level within hesperidin group. In this context, Pari and Suman showed that hesperidin could decrease elevated blood glucose concentration and could increase insulin release in STZ-induced diabetic rats [26]. Akiyama et al. reported that in STZ-induced diabetic rats, hesperidin reduced blood glucose and increased serum insulin levels [30]. It can be hypothesized that the possible mechanism of anti-hyperglycemic action of hesperidin might be through potentiating the pancreatic secretion of insulin from islet β-cells and/or due to enhanced transport of blood glucose to peripheral tissue, or by other mechanisms such as stimulation of glucose uptake by peripheral tissue, inhibition of endogenous glucose production or activation of gluconeogenesis in the liver and muscles [26].

Table 3. Levels of blood glucose, HbA1C, serum insulin, HOMA-IR, anti-inflammatory biomarkers and lipid profile in hesperidin and placebo groups at baseline and the 8th week of study*.

Variables	Group	Before	After	Mean change from base to end	P2
FBG (mg/dl)	Hesperidin	126±15.37	119±15.69	-7±12.99	0.014
	Placebo	138.31± 37.46	131.63± 22.86	-6.68±31.36	0.32
	P1	0.172	0.041	0.941	
HbA1c (%)	Hesperidin	6.53±0.94	6.36±0.83	-0.17±0.56	0.112
	Placebo	6.87±0.84	7±1.06	0.13±0.58	0.335
	P1	0.196	0.028	0.099	
Serum insulin (µU/ml)	Hesperidin	8.13±6.11	9.65±7.65	1.52±0.93	0.018
	Placebo	10.20±7.35	10.15±7.09	-0.04±4.7	1
	P1	0.413	0.725	0.199	
HOMA-IR	Hesperidin	2.56±1.88	2.86±2.20	0.2±0.93	0.117
	Placebo	3.6±2.8	3.36±2.49	-0.24±0.41	0.589
	P1	0.15	0.48	0.246	
hs-CRP (ng/mL)	Hesperidin	2.49±1.92	2.56±4.06	0.06±2.72	0.584
	Placebo	3.14±2.75	3.51±3.72	0.37±4.20	0.807
	P1	0.708	0.641	0.771	
IL-6 (pg/mL)	Hesperidin	8.66±2.08	10.43±12.83	1.77±13.19	0.235
	Placebo	10.33±11.71	8.52±2.63	-1.81±11.93	0.394
	P1	0.224	0.666	0.346	
TC (mg/dL)	Hesperidin	156.47±32.66	148.82±32.40	-7.65±29.30	0.049
	Placebo	169.22±30.97	161.54±42.17	-7.68±32.94	0.261
	P1	0.183	0.255	0.332	
TG (mg/dL)	Hesperidin	157.86±80.62	127.43±82.86	-30.43±83.30	0.132
	Placebo	162.90±22.00	145.72±86.94	-17.18±63.07	0.866
	P1	0.794	0.554	0.552	
HDL (mg/dL)	Hesperidin	38.65±11.62	38.16±9.27	-0.49±4.07	0.593
	Placebo	38.75±11.41	42.85±23.44	4.1±22.27	0.866
	P1	0.794	0.554	0.336	
LDL (mg/dL)	Hesperidin	86.52±20.74	83.32±24.43	-3.2±14.06	0.288
	Placebo	96.59±23.07	94.18±30.46	-2.41±25.13	0.658
	P1	0.143	0.195	0.897	

*Data were expressed as mean±SD, P1 was resulted from independent sample t-test (comparison between the two groups before and after the study), P2 was resulted from paired sample t-test (comparison in each group between before and after values), FBG: fasting blood glucose, HbA1c: glycated hemoglobin, hs-CRP: high-sensitivity C-reactive protein, IL-6: interleukin-6, TC: Total cholesterol, TG: Triglyceride, HDL-C: High-density lipoprotein cholesterol, LDL-C: Low-density lipoprotein cholesterol

By their ability to scavenge free radicals, hesperidin prevents oxidative stress and protects β-cells which may result in increasing insulin secretion and decreasing in elevated blood glucose levels [31]. Changes in concentrations of plasma lipids including cholesterol and lipoprotein are complications frequently observed in patients with diabetes mellitus and certainly contribute to development of coronary heart disease in these patients [32]. In the present study, the serum TC was significantly decreased at the end of the study compared with the beginning of the study in hesperidin group; however, there were no significant differences in other parameters of lipid profile levels between intervention and placebo groups. In agreement with these findings, some animal studies and clinical trials demonstrated that hesperidin or orange juice administration could decrease TC levels [33]. Grinstein et al. found that hesperidin and naringin supplementation significantly lowered TC plasma levels in rats fed a cholesterol-containing diet [34]. Several studies have shown conflicting results associated with the effect of hesperidin on lipid profile [17,35,36]. Yasim and others showed that hesperidin administration in rats fed a high-cholesterol diet increased HDL-C levels significantly, but did not considerably affect other lipid parameters [37]. However, it was observed that hesperidin and its structurally related metabolites lowered plasma cholesterol in rats fed a high-cholesterol diet, accompanied by inhibition of hepatic hydroxymethyl-glutaryl coenzyme A (HMG-CoA) reductase and cholesterol acyl-CoA: cholesterol acyltransferase (ACAT) activities and an increase in fecal acidic sterols [38]. A report by Lee et al. speculated on cholesterol-lowering action of hesperidin via inhibition of HMG-CoA reductase and ACAT activities in rats fed with a high-cholesterol diet [39]. The two key enzymes involved in regulation of cholesterol metabolism are HMG-CoA reductase, a rate-limiting enzyme in cholesterol biosynthetic pathway, and ACAT, a cholesterol-esterifying enzyme in tissues, including the small intestine. Inhibition of HMG-CoA reductase decreases cholesterol synthesis and thus its inhibitors are very effective in lowering serum cholesterol in most animal species, as well as humans [40]. Although in the present study, plasma levels of LDL-C in hesperidin group decreased marginally, this change did not reach the significant level compared to placebo group. Studies regarding the anti-inflammatory effects of hesperidin are very limited and its effect in humans, especially in patients with diabetes, remains unclear. The results from a crossover study conducted in healthy subjects showed that consumption of orange juice or hesperidin for 4 weeks did not affect plasma concentrations of hs-CRP and IL-6 in healthy subjects [41]. Contrary to this finding, one study reported that oral consumption of hesperidin (500 mg once daily for 3 weeks) reduced hs-CRP in subjects with metabolic syndrome [42]. Insulin resistance may be worsened by increased circulating levels of proinflammatory markers including TNF-α and hs-CRP [7]. However, in this study, comparison of groups (before and after the intervention) showed that serum levels of all inflammatory biomarkers did not have any significant changes at the end of the study in both groups.

In our study, no significant changes in systolic or diastolic blood pressure were observed during the intervention. Our results are in consistent with data reported by Wang and colleagues, who showed that consumption of hesperidin in rats fed a high-cholesterol diet, did not lead to a significant change in their blood pressure [38]. This finding contrasts with a study which demonstrated that the consumption of orange juice as well as hesperidin for 4 weeks in healthy subjects resulted in a significant lower diastolic blood pressure compared to that measured after consumption of placebo [41]. The possible mechanisms by which flavonoid-rich foods lower blood pressure may involve in a chronic increase in the production of nitric oxide by vascular endothelium [43]. Other mechanisms, such as an inhibitory effect on angiotensin-converting enzyme, could also be responsible for blood pressure–lowering effects of flavanones [44].

The design of present investigation, namely repeated assessment of diet and physical activity, exclusion of subjects using tobacco or acute inflammatory disease and application of a double-blind, randomized-controlled design by adjusting possible confounders, were all strengths of this study. However our study had some limitations. The sample size was determined on the basis of changes

in IL-6 by considering 80% power and 95% confidence. In the case of this particular variable, the power obtained was sufficient to produce significant results. For some other variables, it seems that higher doses and longer durations of hesperidin administration along with larger sample size and a higher power are needed to attain statistical significance. Furthermore, due to budget limitations, we were not able to measure some markers such as other inflammatory markers, oxidative stress markers such as malondialdehyde, total antioxidant capacity levels and endogenous antioxidants like glutathione.

CONCLUSION

In conclusion, the present study showed that intake of hesperidin could lead to decreased levels of FBG, HbA1c and TC and increased levels of insulin in patients with type 2 diabetes. However, the effect of hesperidin on blood pressure, inflammatory factors and other lipid profile in individuals with diabetes, were not significant in this study. Further researches with long follow up period and high number of participants are needed to reach stronger and more reliable conclusions.

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