Anti-Hyperglycemic Effect of *Vaccinium arctostaphylos* in Type 2 Diabetic Patients: A Randomized Controlled Trial

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Keywords

Patient · Type 2 diabetes · *Vaccinium arctostaphylos*

Summary

Background: Type 2 diabetes mellitus is a common disease. Preliminary data indicate that *Vaccinium arctostaphylos* L. (Caucasian whortleberry) has a potential effect in glycemic control. Thus, the efficacy and safety of a standardized whortleberry fruit hydroalcoholic extract in the treatment of type 2 diabetic patients were studied.

Methods: This randomized double-blind placebo-controlled clinical trial consisted of 37 patients aged 40–60 years with type 2 diabetes who were resistant to conventional oral anti-hyperglycemic drugs. The patients were treated with the whortleberry fruit hydroalcoholic extract (1 capsule = 350 mg, every 8 h for 2 months) in combination with anti-hyperglycemic drugs, and the effects on the blood levels of fasting glucose, 2-hour postprandial glucose, glycosylated hemoglobin (HbA1c), and liver/kidney function were tested, evaluated, and compared with a placebo group (n = 37). **Results:** The extract significantly lowered the blood levels of fasting glucose, 2-h postprandial glucose, and HbA1c (p = 0.007, p < 0.001, and p = 0.005, respectively) without any significant effects on the liver/kidney function (p > 0.05) compared with placebo at the end. No adverse effects were reported. **Conclusion:** Whortleberry may safely improve glycemic control in type 2 diabetic patients.

Schlüsselwörter

Patient · Typ-2-Diabetes · *Vaccinium arctostaphylos*

Zusammenfassung

Hintergrund: Bei Diabetes mellitus Typ 2 handelt es sich um eine weitverbreitete Krankheit. Die hemmende Wirkung von Heidelbeeren (Kaukasische Heidelbeeren / *Vaccinium arctostaphylos*) auf den menschlichen Blutzuckerspiegel ist bereits seit Längerem bekannt. Vor diesem Hintergrund wurden die Effektivität und die Sicherheit der Verabreichung eines hydroalkoholischen Extrakts aus Heidelbeeren unter den an Diabetes mellitus erkrankten Patienten geprüft. **Methoden:** Bei diesem klinischen Versuch wurde die Wirkung von *Vaccinium arctostaphylos* bei 40- bis 60-jährigen Typ-2-Diabetikern untersucht, die nicht auf oral verabreichte, herkömmliche antihyperglykämische Arzneimittel ansprachen. Die Versuchsgruppe (n = 37) wurde alle 8 h über einen Zeitraum von 2 Monaten mit dem hydroalkoholischen Extrakt aus Heidelbeeren (1 Kapsel = 350 mg) in Kombination mit antihyperglykämischen Arzneimitteln behandelt. Die Auswirkungen auf den Blutzuckerspiegel, 2-Stunden-postprandiale Glukosewerte, glykolisiertes Hämoglobin (HbA1c) und die Leber- und Nierenfunktion wurden getestet, evaluiert und mit einer Placebo-Kontrollgruppe (n = 37) verglichen. **Ergebnisse:** Das Heidelbeerextrakt zeigte eine signifikante Wirkung auf den Nüchtern-Blutzuckerspiegel, die 2-Stunden-postprandialen Glukosewerte und den HbA1c (p = 0,007, p < 0,001 und p = 0,005 im Nachhinein). Beeinträchtigungen der Leber- bzw. Nierenfunktionen (p > 0,05) konnten am Ende des Versuchs im Vergleich mit der Placebo-Kontrollgruppe nicht festgestellt werden. Nebenwirkungen wurden nicht berichtet. **Schlussfolgerung:** Heidelbeeren können die glykämische Kontrolle bei Typ-2-Diabetikern ohne Nebenwirkungen stabilisieren.

Introduction

Type 2 diabetes mellitus (T2DM) is a common disease worldwide [1]. Multiple anti-hyperglycemic drugs with different mechanisms are often needed for effective treatment of type 2 diabetic patients [2]. T2DM is characterized by a progressive decline of β -cell function up to its exhaustion, so that an adequate glycemic control with a logical combination of oral therapies cannot be achieved, which leads to the need of insulin as sole therapy. Up to 50% of patients initially treated with oral anti-hyperglycemics ultimately need insulin [3]. Conventional anti-hyperglycemic drugs have limited efficacies and significant adverse effects. Thus, more efficacious and safer anti-hyperglycemic agents are needed [4]. Plants have played a significant role in maintaining human health and improving the quality of life for thousands of years [5]. Herbal supplements may be effective in prevention and treatment of diseases [6–8]. Currently, there is renewed interest in plant-based medicines and functional foods that modulate physiological effects in the prevention and cure of diabetes. The plant kingdom is a vast field to search for natural effective oral anti-hyperglycemic agents that have only slight or no side effects. More than 1,200 plant species are used empirically for their alleged anti-hyperglycemic activity [9]. In Iran, Turkey, and Caucasus, the dried fruit of *Vaccinium arctostaphylos* L. (Caucasian whortleberry, Ericaceae family), hereafter referred to as whortleberry, is taken traditionally at the dose of 5 g/day to treat dia-

betes mellitus and hypertension [10–12]. Whortleberries are rich in anthocyanins [11]. Anthocyanins display a wide range of biological activities including anti-diabetic, anti-obesity, anti-hypertensive, cardioprotective, cataract-preventing, and microcirculation- and vision-improving activities [13–16]. Very little research has been conducted on the anti-hyperglycemic effect of whortleberry. An ethanolic extract of whortleberry fruit was shown to have an anti-hyperglycemic effect in alloxan-diabetic Wistar rats [17]. Further, administration of the whortleberry leaf extract (250 mg every 8 h) to mildly hyperglycemic type 2 diabetic patients without any other anti-hyperglycemic agent lowered blood levels of fasting glucose significantly compared with baseline in a 4-week randomized, double-blind, placebo-controlled clinical trial [12]. However, the effect of whortleberry on the glycosylated hemoglobin (HbA1c) level, as a measure of glycemic control in diabetic patients, as well as its role in the treatment of type 2 diabetic patients resistant to conventional oral anti-hyperglycemic drugs have not been studied so far. Thus, further trials are needed to more precisely define the clinical efficacy and safety of whortleberry in the treatment of diabetes mellitus.

We evaluated the efficacy and safety, compared with placebo, of a whortleberry fruit extract in the treatment of type 2 diabetic patients who were resistant to conventional oral anti-hyperglycemic drugs and thus needed, but were declining, insulin therapy. We also standardized the extract through determining the total anthocyanin content.

Fig. 1. The CONSORT flowchart describing the progress of the patients through the trial.

Material and Methods

Whortleberry

Whortleberry was collected in the Ardebil province of Iran in October 2009 and its identity was authenticated by a botanist (Y. Ajanii). A voucher specimen of the plant (no. 1023) was deposited in the Central Herbarium of the Research Institute of Medicinal Plants. The fruits were separated from the plant, washed, and dried in shade at room temperature. The dried fruits were ground to powder.

Preparation of the Whortleberry Extract for Patients' Use

The dried whortleberry fruit powder (20 kg) was extracted with ethanol/water (70/30) in a percolator for 72 h, and the solvent was removed from the extract in a rotary evaporator. As an excipient, toast powder was added to and mixed with the concentrated extract; the mixture was then ground to a powder. The quantity of the extract powder produced was 5.2 kg. The yield of the extraction process used in this study was 21%; the excipient constituted 19.2% of the extract powder.

Preparation of the Extract Powder and Placebo Capsules

The extract powder as the phytomedicine and toast powder as the placebo were separately filled into oral gelatin capsules with identical appearance by a hand-operated capsule-filling machine (Scientific Instruments and Technology Corporation, USA). The whortleberry capsules contained 350 mg extract powder. The whortleberry and placebo capsules were packed into small indistinguishable labeled containers.

Measurement of Total Anthocyanins

The total anthocyanin content of the extract was measured spectrophotometrically. The extract (1 g) was dissolved in 1.5 N hydrochloric acid in 95% ethanol (15:85 v/v). The solution was filtered through a paper filter into a 250-ml volumetric flask and diluted with solvent to 250 ml. The absorbance was measured at 535 nm. Measurements of total anthocyanins on the sample were replicated 3 times. The results are calculated as: mg total anthocyanins/10 g extract = $(Ab \times 25,000)/98.2$, where Ab = absorption of solution [19].

Table 1. The demographic data of the participants who completed the trial^a

Parameter	Whortleberry group	Placebo group
Age, years	56.6 ± 10.1	53.6 ± 8.9
Gender		
Male	18	21
Female	19	16
Duration of type 2 diabetes, years	7.1 ± 5.4	8.5 ± 3.8
Body mass index, kg/m ²	29.3 ± 7.6	28.9 ± 8.4

^aWhere appropriate, data are given as mean ± standard deviation (SD).

Patients

Inclusion criteria included type 2 diabetic outpatients aged 40–60 years who were resistant to conventional oral anti-hyperglycemic drugs (with fasting blood levels of glucose between 200 mg/dl and 250 mg/dl and HbA1c between 7% and 8% despite using a combination of conventional oral anti-hyperglycemic drugs, including glibenclamide, metformine, glimepiride, acarbose, pioglitazone, and repaglinide) and who needed insulin therapy but refused it.

Exclusion criteria included patients with cardiac, renal, or hepatic diseases or who had concomitant infections, pregnant women, women planning pregnancy, and breast-feeding women.

Protocol

In this trial, 86 Iranian male and female outpatients (42 patients in the whortleberry group and 44 patients in the placebo group) were recruited according to the inclusion and exclusion criteria and randomly received intended treatment in the Diabetes Clinic (Karaj, Iran) from September 1, 2010 to October 1, 2011. 37 patients in each group finished the trial. This sample size was calculated to be sufficient to estimate a 20 mg/dl difference in fasting glucose between the groups, considering type I error = 0.05 and 80% power. The CONSORT flowchart describing the progress of the patients through the trial is shown in figure 1. The demographic data of the subjects who completed the trial are given in table 1.

The whortleberry and the placebo group trials were run concurrently, with each patient taking 1 capsule orally every 8 h for 2 months. The dosage of the extract (350 mg) was based on the anti-hyperglycemic dose of the plant fruit in the traditional medicine (5 g/day) and the yield of the extraction process used in this study (21%). Block randomization with computer-generated random-numbers table and sequentially numbered containers each representing a block consisting of 2 patients were used for treatment allocation. Different persons generated the random allocation sequence, enrolled the participants, and assigned them to interventions. These persons, the care-providers, and the participants were blinded to the interventions. However, as this was not triple-blind trial, the person assessing the outcomes was not blinded to interventions. The study was a randomized double-blind placebo-controlled clinical trial. During the trial, the patients continued to take the conventional oral anti-hyperglycemic drugs that they were taking before, without any dose change. In addition, the patients were recommended to restrict their intake of carbohydrates such as rice, confectionery, pies, and cream pies from 2 months before the beginning of the trial onward. All the subjects recorded the type and amount of the daily consumed foods for 3 days every week. To monitor the patients' compliance with the allocated treatments, the patients returned any capsules left and were asked questions about taking the capsules on their monthly visits. The treatment, diet, and physical activity of the patients completing the trial remained unchanged throughout the study. At the beginning and end of the study, the blood levels of fasting (for 12 h) glucose, 2-hour postprandial glucose, HbA1c, creatinine, and liver enzymes including SGOT (serum glutamic oxaloacetic transaminase) and SGPT (serum glutamic pyruvic

Table 2. Blood parameter levels before and after intervention and their changes during the study

Parameter	Group	Mean (SD) before	p value ^a	Mean (SD) after	p value ^a	Change %, mean (SD)	p value ^a
Fasting glucose	1	191.7 (26.9)	0.1	160.3 (32.3)	0.007	16.3 (11.6) ↓	<0.001
	2	179.8 (34.3)		187.3 (48.8)		4.7 (22.6) ↑	
2-hour postprandial glucose	1	245.8 (37.6)	0.6	209.8 (32.4)	<0.001	13.5 (15.5) ↓	<0.001
	2	249.5 (32.4)		262.8 (28.3)		6.5 (14) ↑	
HbA1c	1	7.6 (0.9)	0.1	6.9 (0.6)	0.005	7.3 (11.8) ↓	<0.001
	2	7.3 (1.1)		7.4 (0.7)		3.3 (12) ↑	

^ap < 0.05, significant (Mann-Whitney U test); ↓ decrease; ↑ increase. Group 1 = whortleberry group; group 2 = placebo group.

Table 3. Blood levels of creatinine, SGPT and SGOT before and after intervention

Parameter	Group	Mean (SD) before	p value ^a	Mean (SD) after	p value ^a
Creatinine	1	0.8 (0.1)	0.1	0.9 (0.1)	0.8
	2	0.9 (0.1)		0.9 (0.1)	
SGPT	1	21.5 (8.3)	0.2	26.7 (9)	0.7
	2	19.3 (6.7)		25.8 (11.4)	
SGOT	1	22 (6.6)	0.3	25.3 (7)	0.1
	2	20.7 (5.7)		22.8 (6.2)	

SGPT = serum glutamic pyruvic transaminase; SGOT = serum glutamic oxaloacetic transaminase; group 1 = whortleberry group; group 2 placebo group.

^aMann-Whitney U test (>0.05, nonsignificant). Group 1 = whortleberry group; group 2 = placebo group.

Fig. 2. The % changes of the blood fasting glucose (FBG), 2-hour postprandial glucose (PPBG) and glycosylated hemoglobin (HbA1c) levels of the whortleberry (group 1) and placebo (group 2) groups. FBGdepercent = % of FBG decrease, PPBGdepercent = % of PPBG decrease, and HA1cdepercent = % of HbA1c decrease.

transaminase) in both the whortleberry and the placebo groups were determined with standard enzymatic kits produced by the Pars Azmoon company (Tehran, Iran) and an auto analyzer (Hitachi 902, Japan). The fasting glucose and HbA1c levels were the primary outcome measures. The other blood parameter levels were the secondary outcome measures. At the end of the study, the baseline homogeneity of the blood parameter means across the whortleberry and placebo groups was analyzed by the Mann-Whitney U test and p values below 0.05 were considered as significant. The data of the patients in the whortleberry and placebo groups were also compared by the Mann-Whitney U test and p values below 0.05 were considered as significant. All statistical analyses were per-protocol analyses. All participants were requested to report any adverse effects. Written informed consent was obtained from the patients. The medical ethics committee of the Research Institute for Islamic and Complementary Medicine approved the protocol (approval number and date: 238/tm/p26 and May 12, 2010). The trial was registered in the Iranian Registry of Clinical Trials with the number IRCT138902292288N3 accessible at www.irct.ir.

Results

Measurement of Total Anthocyanins

The total anthocyanin content was 86.56 ± 2.46 (mean $\pm$ SD) mg / 10 g extract.

Patients

No adverse effects were reported. The groups were matched with regard to demographic data (age, gender, duration of diabetes, and body mass index) (table 1). The baseline blood levels of all parameters were not significantly different between the 2 groups ($p > 0.05$) (tables 2 and 3).

The extract lowered the fasting glucose, 2-h postprandial glucose and HbA1c levels significantly ($p = 0.007$, $p < 0.001$ and $p = 0.005$, respectively) without any significant effects on the other parameter levels ($p > 0.05$) compared with the placebo group at the endpoint. The percentages of endpoint re-

ductions of the fasting glucose, 2-hour postprandial glucose and HbA1c levels in the whortleberry group compared with the baseline levels were 16.3%, 13.5%, and 7.3%, respectively (tables 2 and 3). The percent changes of the fasting glucose, 2-hour postprandial glucose and HbA1c levels of the whortleberry and placebo groups are shown in figure 2.

Discussion

The whortleberry fruit extract lowered the blood levels of fasting glucose, 2-hour postprandial glucose, and HbA1c compared with the baseline at the endpoint; these decreases were significant when compared with the placebo group. The lack of any significant effects of the whortleberry extract on the blood levels of SGOT, SGPT, and creatinine compared with the placebo group demonstrates that whortleberry does not have toxic effects on the hepatic and renal functions. No adverse effects were reported by the patients. The results indicate that whortleberry is a safe anti-hyperglycemic agent that improves glycemic control in type 2 diabetic patients. Whortleberry appears to improve the efficacy of conventional oral anti-hyperglycemic drugs to achieve target glycemic goals and also its own anti-hyperglycemic efficacy is greater than that of the conventional oral anti-hyperglycemic drugs used. As regards the cardiovascular effects of anthocyanins, whortleberry is comparable to metformin. Currently, metformin is the sole conventional anti-diabetic with cardiovascular actions and affects microcirculation independently from its glucose-lowering capacity [19]. The extraglycemic effects of whortleberry may help to reduce long-term complications of T2DM.

The results of the present study are in accord with the studies on the effects of whortleberry in alloxan-diabetic Wistar

rats [17] and type 2 diabetic patients [12]. As far as we know after searching various databases, the bioactives mediating the anti-hyperglycemic effect of whortleberry have not yet been characterized. The only bioactive identified and quantified in the extract used in the present trial was the total anthocyanins. The bioactives and mechanisms involved in the anti-hyperglycemic action of the whortleberry extract were not examined in the current study. However, whortleberry has been shown to contain anthocyanins [11], chlorogenic acid [20], and myricetin [12], which may be involved in the anti-hyperglycemic effects of whortleberry [12, 21–23]. Thus, in view of the results of this study, further trials with larger number of patients assessing the efficacy and safety of whortleberry in the treatment of T2DM and type 1 diabetes mellitus, as well as more studies addressing the mechanisms and bioactives involved in the anti-hyperglycemic action of whortleberry, seem necessary.

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Disclosure Statement

The authors declare that there are no conflicts of interest.

References

- Campbell RK: Type 2 diabetes: where we are today: an overview of disease burden, current treatments, and treatment strategies. *J Am Pharm Assoc* 2009;49:S3–S9.
- Derosa G, Sibilla S: Optimizing combination treatment in the management of type 2 diabetes. *Vasc Health Risk Manag* 2007;3:665–671.
- Massi-Benedetti M, Orsini-Federici M: Treatment of type 2 diabetes with combined therapy: what are the pros and cons? *Diabetes Care* 2008;31: S131–135.
- Krentz AJ, Bailey CJ: Oral antidiabetic agents: current role in type 2 diabetes mellitus. *Drugs* 2005;65:385–411.
- Huang THW, Kota BP, Razmovski V, Roufogalis BD: Herbal or natural medicines as modulators of peroxisome proliferator-activated receptors and related nuclear receptors for therapy of metabolic syndrome. *Basic Clin Pharmacol Toxicol* 2005;96: 3–14.
- Fugh-Berman A: Herbs and dietary supplements in the prevention and treatment of cardiovascular disease. *Prev Cardiol* 2000;3:24–32.
- Tapsell LC, Hemphill I, Cobiac L, Patch CS, Sullivan DR, Fenech M, Roodenrys S, Keogh JB, Clifton PM, Williams PG, Fazio VA, Inge KE: Health benefits of herbs and spices: the past, the present, the future. *Med J Aust* 2006;185:S4–S24.
- Low Dog T: A reason to season: the therapeutic benefits of spices and culinary herbs. *Explore (NY)* 2006;2:446–449.
- Tundis R, Loizzo MR, Menichini F: Natural products as alpha-amylase and alpha-glucosidase inhibitors and their hypoglycemic potential in the treatment of diabetes: an update. *Mini Rev Med Chem* 2010;10:315–331.
- Zargari A: *Medicinal Plants*, ed 4. Tehran, Tehran University Publications, 1997, pp 409–417.
- Latti AK, Kainulainen PS, Hayirlioglu-Ayaz S, Ayaz FA, Riihinen R: Characterization of anthocyanins in Caucasian blueberries (*Vaccinium arctostaphylos* L.) native to Turkey. *J Agric Food Chem* 2009;57:5244–5249.
- Abidov M, Ramazanov A, Jimenez Del Rio M, Chkhikvishvili I: Effect of blueberin on fasting glucose, C-reactive protein and plasma aminotransferases, in female volunteers with diabetes type 2: double-blind, placebo controlled clinical study. *Georgian Med News* 2006;141:66–72.
- Kowalczyk E, Krzesinski P, Kura M, Szmigiel B, Blaszczyk J: Anthocyanins in medicine. *Pol J Pharmacol* 2003;55:699–702.
- Ghosh D, Konishi T: Anthocyanins and anthocyanin-rich extracts: role in diabetes and eye function. *Asia Pac J Clin Nutr* 2007;16:200–208.
- Mazza GJ: Anthocyanins and heart health. *Ann Ist Super Sanita* 2007;43:369–374.
- de Pascual-Teresa S, Moreno DA, Garcia-Viguera C: Flavanols and anthocyanins in cardiovascular health: a review of current evidence. *Int J Mol Sci* 2010;11:1679–1703.
- Feshani AM, Kouhsari SM, Mohammadi S: *Vaccinium arctostaphylos*, a common herbal medicine in Iran: molecular and biochemical study of its antidiabetic effects on alloxan-diabetic Wistar rats. *J Ethnopharmacol* 2011;133:67–74.

- 18 Francis FJ: Analysis of anthocyanins; in Markakis P (ed): Anthocyanins as Food Colors. New York, Academic Press, 1982, pp 182–205.
- 19 Wiernsperger N, Rapin JR: Microvascular diseases: is a new era coming? Cardiovasc Hematol Agents Med Chem 2012;10:167–183.
- 20 Ayaz FA, Hayirlioglu-Ayaz S, Gruz J, Novak O, Strnad M: Separation, characterization, and quantitation of phenolic acids in a little-known blueberry (*Vaccinium arctostaphylos* L.) fruit by HPLC-MS. J Agric Food Chem 2005;53:8116–8122.
- 21 Matsui T, Ebuchi S, Kobayashi M, Fukui K, Sugita K, Terahara N, Matsumoto K: Anti-hyperglycemic effect of diacylated anthocyanin derived from Ipomoea batatas cultivar Ayamurasaki can be achieved through the alpha-glucosidase inhibitory action. J Agric Food Chem 2002;50:7244–7248.
- 22 Van Dijk AE, Olthof MR, Meeuse JC, Seebus E, Heine RJ, van Dam RM: Acute effects of decaffeinated coffee and the major coffee components chlorogenic acid and trigonelline on glucose tolerance. Diabetes Care 2009;32:1023–1025.
- 23 Ong KC, Khoo HE: Effects of myricetin on glycemia and glycogen metabolism in diabetic rats. Life Sci 2000;67:1695–1705.