Effects of *Rosa canina* L. Fruit on Glycemia and Lipid Profile in Type 2 Diabetic Patients: A Randomized, Double-Blind, Placebo-Controlled Clinical Trial

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Abstract

Background: ***Rosa canina*** **L. (rose hip) has been traditionally used to treat diabetes mellitus in Iran. However, no scientific human study has determined its efficacy in diabetic patients.**

Objective: This study was conducted to evaluate the efficacy and safety of *R. canina* fruit aqueous extract in type 2 diabetic patients.

Methods: Sixty patients with type 2 diabetes, aged 35 - 60 years with fasting blood glucose levels between 130 to 200 mg/dL and HbA1c between 7 - 9% despite using conventional oral hypoglycemic drugs were divided randomly to two groups. Two groups of 25 and 23 patients completing the trial received 750 mg *R. canina* fruit extract and 750 mg toast powder as placebo two times a day respectively for three months.

Fasting blood glucose (FBG) and glycosylated hemoglobin (HbA1c) as primary outcomes and postprandial blood glucose (PBG), lipid profile and hepatic and renal function tests as secondary outcomes were determined at baseline and at endpoint of treatment. The patients were asked to note down any gastrointestinal or other side effects during the study.

Results: The FBG level decreased significantly (P = 0.002) in *R. canina* group after 3 months compared to the baseline. In addition total cholesterol/HDL-C was significantly (P = 0.02) decreased in the *R. canina* group compared to the baseline. Other blood parameters were not significantly changed during the study compared with placebo and baseline. No serious side effects were reported in both groups during the study.

Conclusion: *Rosa canina* 3-month administration to type 2 diabetic patients may reduce fasting blood glucose and total cholesterol/HDL-C without any side effect.

Keywords: ***Rosa canina*****, Diabetes mellitus, Herbal medicine, Traditional medicine**

Introduction

Type 2 diabetes mellitus (T2DM) is a common disease worldwide. Between 2010 and 2030, there will be a 69% increase in numbers of adults with diabetes in developing countries and a 20% increase in developed countries [1]. The prevalence of diagnosed and undiagnosed diabetes mellitus in Iran is estimated as 7.7% in people aged 25-64 years [2].

The complications are the main causes of morbidities and mortalities due to T2DM and tight glycemic control is necessary for the management of type 2 diabetes [1].

While insulin and oral anti-hyperglycemic drugs are cornerstone of T2DM treatment, they have important adverse effects and limited efficacy [3]. Thus, more efficacious and safer anti-hyperglycemic agents are needed [4]. Plant-based medicine is the most frequently used type of complementary/alternative medicine for prevention and treatment of several diseases [5-7]. Although more than 1200 plants species have been recorded to be used empirically worldwide for their alleged anti-hyperglycemic activity, there are inadequate clinical trials for evaluating their efficacy and safety in human [8-10].

In Iran (especially in Azerbaijan province), the dried fruit of *R. canina* L. (rose hip, Rosaceous family), is taken traditionally to treat diabetes mellitus. *R. canina* fruits are also used for treatment of colds, scurvy, fever, rheumatic, urinary tract and kidney diseases [11]. A number of studies reported that, *R. canina* fruit, with its high ascorbic acid, phenol and flavonoids contents, have antioxidant, antimutagenic, anticarcinogenic, anti-inflammatory and antinociceptive effects [12-16]. In a study conducted in Turkey, the ethanol extract of *R. canina* fruits and its fractions were screened for their antioxidant, hypoglycemic and antidiabetic activities. A remarkable hypoglycemic effect at the dose of 250 mg/kg of the ethanol extract was reported in streptozotocin induced diabetic rats [17]. Anderson et al. [18] also investigated the possible metabolic effects of rose hip powder by administering as dietary supplement to obese C57BL/6J mice. Improved glucose tolerance, total plasma cholesterol and antidiabetic effects were observed. We performed the present randomized double-blind clinical study to evaluate the efficacy and safety of the *R. canina* fruit aqueous extract in patients with type 2 diabetes.

Materials and Methods

Preparation of the plant extract

The fruits of *R. canina* were purchased from a local herbal store in Maragheh (Azarbaiijan, Iran) and identified by Dr Gholamreza Amin (Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran). The voucher specimen "PMP-636" was deposited in the herbarium of Faculty of Pharmacy, Tehran University of Medical Sciences Tehran Iran. Rose hip powder was extracted with distilled water and concentrated to obtain dry powder. The percentage yield was 31%.

Measurement of total flavonoids content *R. canina* fruits

The total flavonoids content (TFC) in aqueous extract of *R. canina* fruits was determined using $AlCl_3$ reagent [19]. Briefly, 2.5 mL of each sample (and/or quercetin as the standard), previously dissolved in 90% ethanol, was mixed with 2.5 ml of a 2% $AlCl_3$ solution in 90% ethanol. After 40 minute, the absorbance of the yellow color produced was measured at 415 nm. The TFC [as μg quercetin equivalents/mg of sample] for the sample was

calculated on the basis of a linear calibration curve obtained using quercetin ($y = 0.0169x+0.3526$, $r^2 = 0.995$).

Preparation of *R. canina* and placebo capsules

The *R. canina* extract powder (750 mg) as the phytomedicine and toast powder (750 mg) as the placebo were filled into hard oral gelatin capsules with identical appearance. The placebo and *R. canina* capsules were packed into indistinguishable labeled containers. The dosage of the extract (750 mg b.i.d) was based on the minimum dose of rose hip in the folk medicine (5g in a cup of hot water) and the yield of the extraction process (30 %) used in this study.

Patients

Inclusion criteria: type 2 diabetes mellitus outpatients aged 35-60 years with fasting blood levels of glucose between 130 to 200 mg/dL and HbA1c between 7 to 9% despite using a combination of conventional oral hypoglycemic drugs (including metformine and glibenclamide). Exclusion criteria: patients receiving insulin or other hypoglycemic agents, patients with cardiac, renal or hepatic diseases, pregnant women, women planning pregnancy and breast-feeding women.

Protocol

This randomized double blind placebo controlled trial was conducted from March 2012 to June 2013 in the Omid Hospital of the Abhar city in the Zanjan province. The medical ethics committee of the Research Institute for Islamic and Complementary Medicine approved the protocol (approval number and date: 854/tm/p26 and september 2011). The trial was registered in the Iranian Registry of Clinical Trials with the number IRCT138803061957N2.

The sample size was calculated as 30 patients in each group as to estimate 20 mg/dL difference of FBG between the groups, considering type I error = 0.05, 80% power. This sample size was also adequate for estimating 30 mg/dL difference of PPG and 1% difference of HbA1c between the groups. The CONSORT flow diagram in Figure 1. describes the progress of the participants through the trial.

Written informed consent was obtained from all the patients. The patients were randomly allocated to two groups using block randomization method. Blocks volume was 4, blocks selection was based on a computerized list of random numbers and number of patients and type of their interventions were written on a paper and put in an envelope. The study was double-blind. Each patient took one capsule orally every 12 hour for 3 months. The patients were advised to take their conventional oral hypoglycemic agents without any dose changes and not change their diet and physical activity during the study.

To monitor the patients' compliance with the medications, they were asked questions about taking the capsules on their monthly follow up (by telephone).

At the beginning and end of the study, the blood levels of fasting (for 10 h) glucose (FBG), 2-hour postprandial glucose, blood urea nitrogen, creatinine, serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT) were determined with standard enzymatic kits (Pars Azmoon company, Tehran, Iran) using an Auto analyzer (Hitachi 902, Japan). Glycosylated hemoglobin (HbA1c) was measured by high-performance liquid

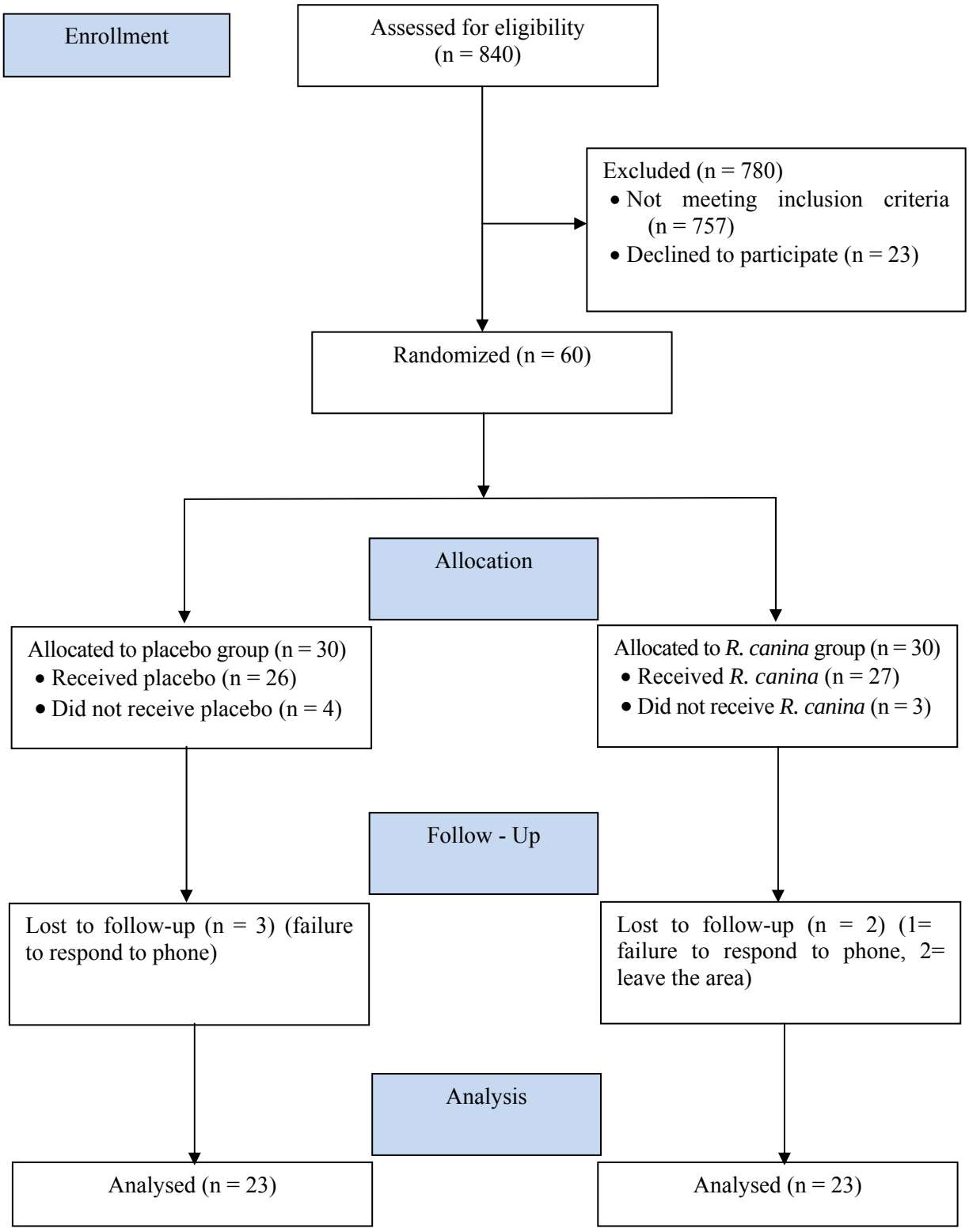

Figure 1- The consort flow diagram of participants

chromatography. The primary outcome measures were the FBG and HbA1c. The other parameters were considered as the secondary outcome measures. All participants were requested to report any adverse effects. The SPSS software v17.0 was used for statistical analyses. Continuous data were described as mean ± standard deviation (SD) and analyzed using the two-sided paired t-test. Nominal variables were described using frequency counts and compared by treatment assignment using the two-sided chi-squared test. *P* values < 0.05 were considered as significant.

Results

The total flavonoids contents of the aqueous extract of *R. canina* fruits as µg of quercetin equivalents in mg of sample was 14.54 ± 0.67 µg/mg (mean ± SD). Out of sixty patients who were screened and randomized, 48 (25 in *R. canina* and 23 in placebo groups) patients completed the study and their data were analyzed. Demographic and baseline characteristics of patients in *R. canina* and placebo groups are summarized in table 1.

The frequency of gender, age, duration of diabetes, BMI, type and dose of oral hypoglycemic drugs were similar between the groups.

Blood parameters at baseline and post treatment are summarized in Table 2.

The FBG level was decreased in the *R. canina* group significantly ($P = 0.002$) after 3 months compared to base line. The PPG and HbA1c levels were not changed significantly in both groups. There were no significant changes in the total cholesterol, triglyceride, LDL-C/HDL-C ratio, BUN, creatinine, SGOT and SGPT levels in both groups after 3 months, but cholesterol/HDL-C ratio was decreased significantly ($P = 0.02$) in the *R. canina* group after 3 months compared to base line. The means of FBG, PPG, HbA1c and cholesterol/HDL ratio before and after *R. canina* and their 95% confidence intervals are presented in table 3.

Table 1- Demographic and characteristics of the patients in the *R. canina* fruits and placebo groups at baseline

	Group*	N
Gender	1	9 male, 16 female
	2	12 male, 11 female
		Mean (± SD)
Age (year)	1	53.7 (9.1)
	2	58.7 (8.5)
Duration of diabetes (year)	1	5.5 (6)
	2	4.9 (4.5)
Body mass index (kg/m2)	1	31.7 (5.2)
	2	30.6 (3.9)
Dosage of oral hypoglycemic drugs (mg/day)	1	12.5 (6) glibenclamide 1150 (435) metformin
	2	10 (9) glibenclamide 1250 (425) metformin
FBG (mg/dL)	1	158.2 (25.6)
	2	147.7 (29.7)
PPG (mg/dL)	1	219.5 (36.9)
	2	222.4 (50.9)
HbA1c (%)	1	8 (0.9)
	2	7.9 (0.6)

*Group1=*Rosa canina* L., Group 2= Placebo

Table 2- Blood parameters levels of the *R. canina* fruits and placebo groups at baseline and post treatment

		R. canina (n = 25)	*P*-value	Placebo (n = 23)	*P*-value
FBG (mg/dL)	pre	157.9 (23.1)	0.002*	144.8 (41.9)	0.8
	post	132.25 (29.2)		142.5 (58.3)	
PPG (mg/dL)	pre	222.1 (29.2)	0.053	198.2 (54.7)	0.6
	post	188.6 (55)		191.2 (60.5)	
HbA1c (%)	pre	8.1 (1)	0.19	7.9 (0.7)	0.3
	post	7.7 (1.1)		7.7 (0.9)	
Triglyceride (mg/dL)	pre	198 (93.3)	0.4	199.6 (112)	0.5
	post	185.8 (113.2)		184.1 (64.5)	
Total cholesterol (mg/dL)	pre	191.2 (43)	0.1	176.1 (33.3)	0.08
	post	171 (32.2)		167.3 (30.8)	
HDL-C (mg/dL)	pre	42.6 (4.8)	0.1	43.4 (3.6)	0.4
	post	44.9 (5.9)		44.5 (5.1)	
LDL-C (mg/dL)	pre	110.3 (44)	0.4	99.4 (36.2)	0.07
	post	99.5 (39.1)		86.2 (29.3)	
Cholesterol/HDL-C	pre	4.6 (1)	0.02*	4.2 (0.8)	0.2
	post	3.8 (0.8)		3.8 (1)	
LDL-C/HDL-C	pre	2.5 (1)	0.4	2.3 (0.9)	0.2
	post	2.2 (1.1)		2.1(0.8)	
BUN (mg/dL)	pre	27.9 (9.2)	0.1	29.2 (11.5)	0.2
	post	26.4 (9.5)		31.3 (12.3)	
Creatinine (mg/dL)	pre	0.9 (0.2)	0.5	0.9 (0.1)	0.8
	post	0.9 (0.1)		0.9 (0.2)	
SGOT (u/L)	pre	22 .4 (8.9)	0.5	21.5 (6.8)	0.2
	post	23.5 (11.6)		23.5 (10.4)	
SGPT (u/L)	pre	24.5 (13.1)	0.5	27.2 (12)	0.06
	post	25.4 (12.3)		23.6 (7.6)	

All parameters are described as mean (± SD). FBG: fasting blood glucose, PPG: postprandial blood glucose, BUN: blood urea nitrogen, SGOT: serum glutamic oxaloacetic transaminase, SGPT**:** serum glutamic pyruvic transaminase, pre: baseline, post: post treatment. * Statistically significant

Table 3- Paired means of FBG, PPG, HbA1c and cholesterol/ HDL-C in *R. canina* group

	Paired means before and after *R. canina*		
	Mean	**Standard deviation**	**95% confidence interval**
FBG (mg/dL)	25.6	25.5	11.55 − 39.8
PPG (mg/dL)	33.5	61.3	-0.43 − 67.46
HbA1c (%)	0.3	1.1	-0.18 − 0.86
Cholesterol/HDL-C	0.8	1.3	0.13 − 1.42

R. canina and placebo were well tolerated by the patients. There were no serious adverse effects in both groups and no attrition was seen due to side effects. The most common adverse effects in *R. canina* group were self-limiting mild stomach ache and nausea (n = 1), transient diarrhea (n = 1) and weakness (n = 1) at the beginning of consumption. Two of the patients in the placebo group also reported transient diarrhea.

Discussion

The results suggest that *R. canina* may reduce fasting blood glucose and total cholesterol/HDL-C ratio in type 2 diabetic patients without any hepatic, renal or gastrointestinal adverse effects. Although the hypoglycemic effects of *R. canina* fruit were reported in diabetic rat [17-18], the fasting blood glucose lowering effects observed in present study is in contrast to previous clinical trial conducted on obese patients in which 40 gram per day *R. canina* fruit intake did not influence blood glucose levels during 6 weeks study [20]. These contradictory results may be due to limitations of that study; as that study was cross-over and conducted on total 31 diabetic and non diabetic obese patients [20]. In the present study although lipids levels were not affected by *R. canina* fruit, but reduction of cholesterol/HDL-C ratio indicates positive clinical efficacy on lipid profile. The mechanism and bioactive mediating the glycemic effects of *R. canine* is not yet characterized. Anderson et al. [18] proposed that down regulation of the hepatic biogenic program is at least one mechanism underlying the antidiabetic effect of rose hip in C57BL/6J mice fed a high-fat diet.

The effects on glucose and lipid profile observed in the present study in part may be due to phenolic compounds present in rose hip [12]. Flavonoids with anti-oxidant properties indirectly decrease oxidized LDL-C and improve insulin receptor activity [21-22]. In addition, rose hip contains diverse bioactive components such as vitamin C, quercetin, ursolic acid, betulinic acid, oleanolic acid, licopene, linoleic acid, α- linoleic acid and lutein [23-24]. Most of these biochemical components show antidiabetic effects [25-27]. The main limitations of the present study were small sample size and selection of single dose therapy with lowest average doses used in the folk medicine. However, the insignificant changes in HbA1c and lipid levels observed in *R. canina* fruit group may be due to small sample size or low *R. canina* fruit doses used in present study.

Conclusion

In conclusion, 3- month *R. canina* fruit administration to type 2 diabetic patients may reduce fasting blood glucose and total

cholesterol/HDL-C without any side effect**.** Thus, considering the results of the present and previous trials, further larger clinical trials concerning the safety and efficacy of higher dose of *R. canina* fruit in the treatment of patients with T2DM and/or hyperlipidemia as well as more studies addressing the bioactive and mechanisms involved in the anti-hyperglycemic and antihyperlipidemic actions of *R. canina* seem necessary.

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