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Effects of Iranian propolis on glycemic status, inflammatory factors, and liver enzyme levels in type 2 diabetic patients: a randomized, double-blind, placebo-controlled, clinical trial

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Objective: Propolis is one of the hive products with a wide clinical usage due to the variety of bioactive components. This clinical trial was conducted to evaluate the effects of propolis supplementation on glycemic status and inflammation.

Methods: In an 8-week randomized, double-blinded, placebo-controlled, clinical trial, patients with type 2 diabetes were randomly assigned to the propolis (n = 31) or control group (n = 31). The first group received propolis capsules (500 mg), 3 times a day. Fasting blood samples were obtained. The liver enzymes, inflammatory markers, and glucose-related indicators were measured at the beginning and the end of the study.

Results: Compared with the control group, the propolis group showed significant changes in fasting plasma glucose (propolis, -19.8 ± 29.16; placebo, 0.7 ± 27.8; p = 0.01), two-hour postprandial glucose (propolis, -27.42 ± 44.5; placebo, -0.95 ± 42.7; p = 0.001), hemoglobin A1c (propolis, -1.07 ± 1.6; placebo, 0.03 ± 1.5; p = 0.041), insulin (propolis, -1.65 ± 4.3; placebo, 0.04 ± 4.02; p = 0.03), HOMA-IR (propolis, -1.08 ± 0.7; placebo, 0.03 ± 0.42; p = .044), TNF-α (propolis, -2.67 ± 4.1; placebo, 0.12 ± 4; p = 0.025), and C-reactive protein (propolis, -2.5 ± 3.01; placebo, -0.67 ± 2.84; p = 0.031). Furthermore, propolis reduced the mean aspartate aminotransferase (AST) (propolis, -1.62 ± 10.4; placebo, 0.13 ± 11.07; p = 0.12) and alanine aminotransferase (ALT) levels (propolis, -0.61 ± 6.47; placebo, 0.12 ± 7.01; p = 0.54), but it was not significant.

Conclusion: Propolis treatment in type 2 diabetic patients had a beneficial effect on the glycemic profile and inflammatory status. However, there was no significant change in the level of AST and ALT enzymes, warranting further research.

Introduction

Diabetes mellitus (DM) is a common metabolic disease characterized by insufficient insulin activity that results in elevated blood glucose levels. Uncontrolled blood glucose level causes metabolic disorders and micro- and macro-vascular complications [1].

Research has established the relationship between poor blood sugar control and elevated oxidative stress. Oxidative stress has an important role in the pathogenesis of diabetes and causes micro- and macro-vascular complications [2]. Therefore, a balance between antioxidants and diabetes-associated

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oxidative stress is of particular importance. Currently, insulin and various drugs are prescribed for diabetes treatment, which can cause problems for patients because of the wide and long-term consumption [3]. Thus, most of the researchers' interest has been focused on the development of alternative medicinal foods, which include natural bioactive compounds with the ability to improve glucose control and reduce the complications [4].

Propolis is a resinous hive mater made by bees using the pollen from different plant sources in mixture with beeswax and enzymes [5]. It contains active compounds such as flavonoids, terpenes, and phenolic acids, as well as protein, sugar, vitamins, and abundant minerals [6]. Therefore, it possesses a wide spectrum of biological properties, including anti-inflammatory [7-9], antioxidant [10], antiviral and antibiotic [11], antifungal [12], antiatherogenic [13], and anticancer [14]. It has traditionally been used in the prevention and treatment of various diseases, including the treatment of wounds, rheumatism, heart disease, and diabetes [15-17].

In recent years, many studies have investigated the hypoglycemic and antioxidant effects of propolis, mostly in experimental models of diabetes, and have demonstrated potential positive effects on glucose metabolism and antioxidant function in diabetic rats. However, the results are not unequivocal. For example, although Yajing Li et al. demonstrated that encapsulated propolis could improve blood glucose levels, lipid metabolism, and insulin sensitivity in rats with type 2 diabetes mellitus (T2DM) [5], Zhao et al found no significant difference in serum glucose, glycosylated hemoglobin, insulin, adiponectin, and aldose reductase between the propolis and placebo group after an 18-week intervention [18].

Thereby, effects of propolis on metabolic factors in T2DM are uncertain and need further studies. In this study, we investigated the effects of propolis on the glycemic control, serum liver enzyme levels, and inflammation cytokine in T2DM patients.

Methods

Subjects

Patients from Velayat Hospital of Qazvin University of Medical Sciences voluntarily participated in this double-blind randomized controlled trial conducted from 2016 to 2017.

Inclusion and exclusion criteria

Patients were included if they had T2DM, defined as a fasting plasma glucose (FPG) of 126 mg/dl, were 30 to 55 years old, had no change in medications over the past 2 months, and had moderate physical activity levels. At the beginning of the trial, data including age, blood parameters, and medical history were collected using a questionnaire and blood samples. Exclusion criteria were as follows: taking insulin, having diabetes for more than ten year, pregnancy or lactation, hospitalization during the trial, having severe renal or hepatic failure or any serious disease that may affect study (e.g., coronary heart disease, lung disease, kidney disease, or cancer), changing the dose of glucose-lowering drugs, diet, or physical activity level, taking any dietary supplement from 2 months prior to the trial to the end of the trial, having a history of any kind of allergy, smoking, alcohol consumption, travel, reporting any side effects of the intervention.

Anthropometric measurements

The weight measurement was done without shoes and with the minimum possible clothing using the Seca scale with an accuracy of 0.1 kg. Also, height measurements were made by using a height gauge to the nearest 0.1 cm. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters.

Study design

Before participating in the study, informed consent was obtained from all patients. The study was approved by the Ethics Committee of the Qazvin University of Medical Sciences and registered with the clinical trials registry of Iran (IRCT2017041019669N4). Patients were randomly divided into a control (n = 31) or treatment (n = 31) group using a table of random numbers. Patients in the treatment group took 500-mg propolis capsules 3 times per day (with food) for 8 weeks, while the control group received capsules of the same appearance containing 500 mg of wheat flour. The capsules were distributed to the participants according to the allocation codes after randomization. To maintain blinding, the allocation was performed by an investigator with no clinical involvement in the study. The participants were asked to have their usual physical activity and diet and make no change in them.

To control the participants' adherence to the trial protocol and prevent participant loss, follow-up calls were made every week. Treatment adherence was determined by counting the remaining number of capsules at the end of the trial, and patients who had not consumed more than 10% of their supplements were excluded from the study.

Biochemical measurements

Baseline and postintervention venous blood samples (10 ml) were collected between 7 and 9 a.m. after an overnight fast. Fasting blood sugar (FBS) concentration was measured through an enzymatic method with a biochemistry analyzer (Alcyon 300, Abbott Laboratories, USA) using Pars-Azmone kit (Tehran, Iran). HbA1c (%) was determined using an automated high-performance liquid chromatography

analyzer (D-10, Bio-Rad Laboratories, Schiltigheim, France). Plasma insulin was measured using a chemiluminescent immunoassay method (Liaison® Analyzer, DiaSorin, S.p.A., Vercelli, Italy). HOMA-IR was calculated according to the following formula: HOMA-IR = (fasting insulin $_{(U/ml)}$ × FPG $_{(mg/dl)}$) / 405.

C-reactive protein (CRP) concentration was determined using an immune turbid metric assay (Pars Azmoon Co., Tehran, Iran). TNF-α levels were measured with an ELISA kit (bioscience).

Blood factors including alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined by an automated biochemical analyzer (Hitachi-7180E, Tokyo, Japan) with a Pars Azmoon reagent kit (Tehran, Iran).

Statistical analyses

The results are expressed as mean (SD). Data were analyzed using SPSS version 20 software. The normality of the distribution of data was evaluated with the one-sample Kolmogorov-Smirnov test. Data analysis was done by using a paired-samples t-test and independent-samples t-test. The percentage of mean changes of markers was calculated as (8-week values – baseline values) / baseline values × 100. A p value of less than 0.05 was considered statistically significant.

Results

Seventy-two patients were assessed for eligibility for inclusion in the study, of whom 62 were enrolled and randomized to the treatment or control group. One patient from each group dropped out of the study for personal reasons; therefore, data on 30 participants in each group were used in final analysis (Figure 1).Their data were excluded from the final statistical analysis.

diabetes were 69.48 ± 21.4 kg, 26.76 ± 3.35 kg/m2, 1510.21 ± 370.05 mg, and 5.42 ± 3.35 years (Table 1). There were no significant differences in any of the variables at baseline.

Effects of propolis on mean changes and comparison between groups at 8 weeks

Table 2 shows the mean changes after 8 weeks of intervention. There was a significant difference in the mean FBS changes between the propolis and placebo groups. It means that the 8-week treatment with propolis could decrease the level of FBS. However, there was no significant change in FBS in the placebo group. Also, significant differences were observed in the mean changes in 2-hp ($p < 0.001$), insulin ($p < 0.03$), HOMA-IR ($p < 0.04$), and HbA1c ($p < 0.04$) between the two groups, indicating that

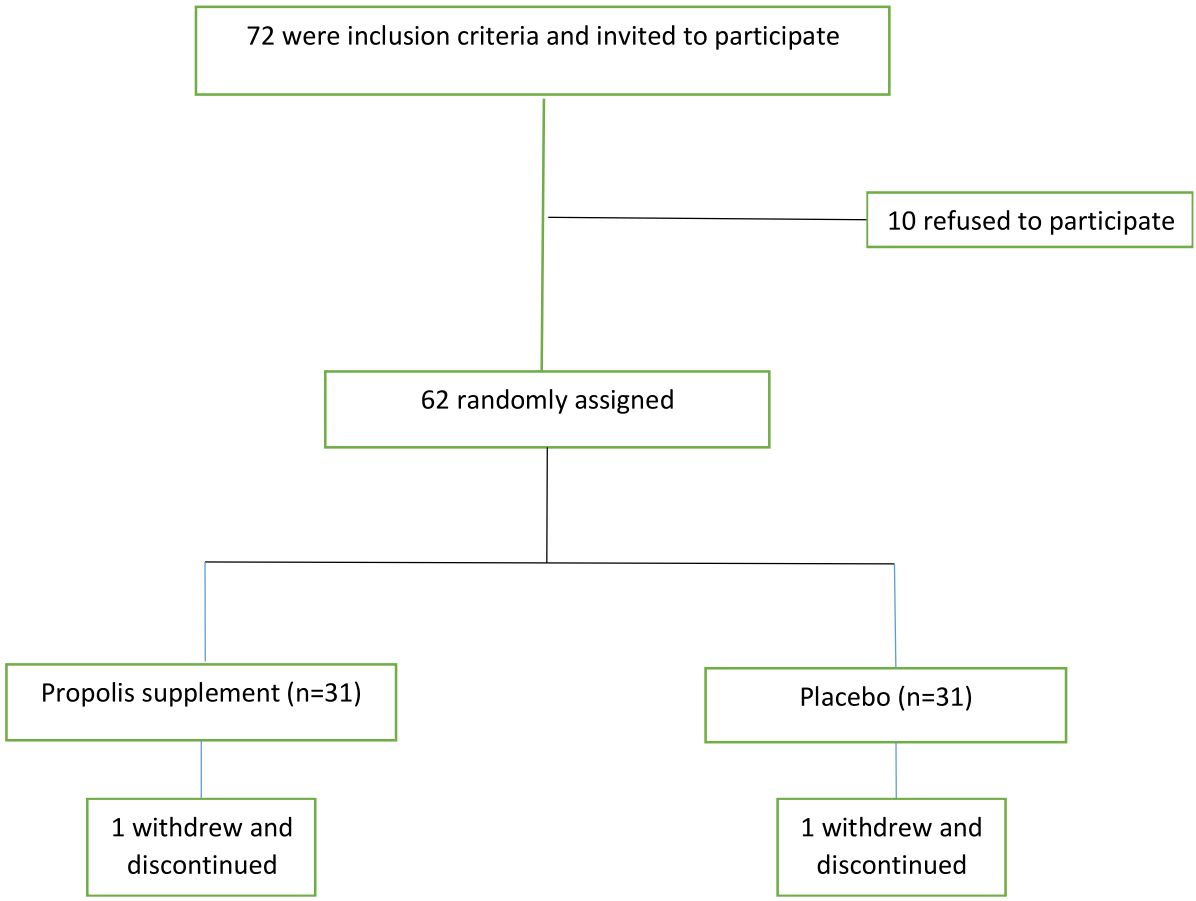

Figure 1. Trial profile and design.

Table1. Baseline characteristics of the participants

Characteristics		Mean ± SD	Mean ± SD	p value
		Propolis (n = 30)	Placebo (n = 30)	
Age (y)		51.81 ± 6.35	49.05 ± 8.2	0.24
Weight (kg)	Initial	68.2 ± 9.7	70.76 ± 11.7	0.63
	End	68 ± 9.04	71.5 ± 11.84	0.42
BMI (kg/m^2)	Initial	26.78 ± 3.01	26.74 ± 3.7	0.81
	End	26.7 ± v2.8	27.01 ± 3.7	0.62
Metformin dose		1518.17 ± 329.2	1502.26 ± 410.91	0.91
Diabetes duration		5.47 ± 3.6	5.38 ± 3.1	0.9

SD, standard deviation; BMI, body mass index; FBS, fasting blood sugar

propolis had a significant effect on glucose metabolism in T2DM patients. Within-group comparisons revealed significant reductions in CRP and TNF-α levels in the propolis group, but not in the placebo group. The difference between the two groups was significant. Propolis treatment also caused a nonsignificant decrease in liver enzyme levels.

No side effects of the treatment with propolis were reported during the trial.

Discussion

The present study investigated the effects of propolis on glucose metabolism, liver enzymes, and inflammatory markers CRP and TNF-α in patients with T2DM. The results showed that propolis treatment significantly lowered FBS, 2-hp, and HbA1c in diabetic patients at the end of the study. Furthermore, daily intake of 1500 mg of propolis for 8 weeks significantly decreased the inflammatory biomarkers.

The biological action of propolis stems from its active constituents, including flavonoids, which possess antioxidant properties [19]. In T2DM, patients have insulin resistance or deficiency of insulin secretion, which causes chronic hyperglycemia and impaired carbohydrate, lipid, and protein metabolism and ultimately leads to the generation of reactive oxygen species and oxidative stress [20, 21].

Several antioxidants, including vitamins and flavonoid compounds, have been investigated for their effects in the treatment of oxidative stress in T2DM, which have demonstrated beneficial effects [22]. For example, Jarouliya et al demonstrated that spirulina improved both blood glucose levels and oxidative markers [23]. Furthermore, T2DM may activate the mitogen-activated protein kinase (MAPK) signaling cascade via ROS production. MAPK is the main component of the proapoptotic signaling pathway, and its disproportionate activation can have destructive effects on cellular function. Sadek and colleagues showed that spirulina did have a significant effect on MAPK activity [24].

Propolis, owing to its powerful antioxidant properties, favorably affects diabetes-associated metabolic abnormalities. In support of this claim, a clinical study reported that propolis reduced blood glucose and lipid levels in patients with T2DM [15]. Also, encapsulated propolis was reported to be effective in controlling blood glucose, modulating lipid metabolism, and improving insulin sensitivity in T2DM rats [5]. However, one study failed to observe significant differences in serum glucose, glycosylated hemoglobin, or insulin between the placebo and treatment groups after 18 weeks of treatment with propolis [18].

On the other hand, diabetes can cause histological

Table 2. Mean changes in outcomes after 8 weeks of treatment

Variables	Mean ± SD	Mean ± SD	p value
	Propolis (n = 30)	Placebo (n = 30)	
FBS (mg/dL)	-19.8 ± 29.16	0.7 ± 27.8	0.01
2-hp (mg/dL)	-27.42 ± 44.5	-0.95 ± 42.7	0.001
Insulin (μU/ml)	-1.65 ± 4.3	0.04 ± 4.02	0.03
HOMA-IR	-1.08 ± 0.7	0.03 ± 0.42	0.044
HbA1c (%)	-1.07 ± 1.6	0.03 ± 1.5	0.041
TNF-α (pg/ml)	-2.67 ± 4.1	0.12 ± 4	0.025
CRP (ng/ml)	-2.5 ± 3.01	-0.67 ± 2.84	0.031
AST (U/L)	-1.62 ± 10.4	0.13 ± 11.07	0.12
ALT (U/L)	-0.61 ± 6.47	0.12 ± 7.01	0.54

and biochemical damages to the liver through ROS-associated oxidative stress, which is demonstrated by elevated levels of aspartate aminotransferase and alanine aminotransferase enzymes and histological damage [25]. However, there is evidence that propolis had positive effects on histopathological and biochemical parameters of liver owing to its antioxidant and anti-inflammatory properties. Kismet et al showed the positive effects of propolis on ALT, alkaline phosphatase, and TNF-α levels in rats with alcoholic fatty liver disease [26]. Our data showed that treatment with propolis reduced AST and ALT enzymes, although not significantly, which may be due to the insufficient dosage of propolis.

Antioxidant activity of propolis is in part attributable to its bioactive components. A clinical study showed that propolis was able to decrease the levels of thiobarbituric acid reactive substances, which are formed as a byproduct of lipid peroxidation, and increase glutathione levels, a powerful antioxidant that protects the important cellular components against free radicals. The study showed that propolis reduces the production of ROS, and consequently oxidative stress, by inhibiting lipid peroxidation [27]. Reports indicate that increased levels of markers and mediators of inflammation and acute-phase reactants such as CRP and IL-6 are correlated with incident T2DM [27-33]. In addition, proinflammatory cytokines such as TNF-α could actually cause insulin resistance in experimental models [34-36]. We showed that propolis reduced insulin resistance by decreasing CRP and TNF-α level.

There were several limitations to this study. First, the inadequate dosage of propolis and period of treatment may have influenced the treatment effects on liver enzymes. Another limitation of this study was that more inflammatory factors should have been investigated, which is expected to be done in future studies.

Conclusion

The results showed that propolis treatment has a beneficial effect on FBS, 2-hp, HbA1c, inflammatory markers, ALT, and AST levels in T2DM patients and may be used as a complementary therapy for type 2 diabetes.

Disclosure statement

There are no competing financial interests.

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