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Effectiveness of Hydroalcoholic Extract Powder Combination of Six Anti-Diabetic Plants Used in Persian Medicine on Blood Glucose Control in Type 2 Diabetic Patients: A Randomized Double-Blind Placebo-Controlled Clinical Trial

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ABSTRACT

In Persian Medicine, herbal therapists prescribe several herbs with different combinations for the treatment of diabetic patients. Among these plants, an herbal combination including *Silybum marianum* (L.) Gaertn. (silymarin), *Melissa officinalis* L. (lemon balm), *Vaccinium arctostaphylos* L. (Caucasian whortleberry), *Trigonella foenum-graecum* L. (fenugreek), *Urtica dioica* L. (nettle), *and Citrullus colocynthis* (L.) Schrad. (colocynth) is prescribed for treatment of diabetic patients by some herbalists. The efficacy and safety of this herbal combination were evaluated in animal and cell culture studies in the Institute of Medicinal Plants. However, no clinical studies have been conducted to confirm its clinical safety and effectiveness. The present study was performed to investigate the efficacy and safety of this herbal combination on the blood glucose control in type 2 diabetic patients. The herbal combination and placebo capsules (500 mg) were provided by the Institute of Medicinal Plants. Sixty eligible type 2 diabetic patients referred to the Diabetes Research Center affiliated to the Alborz University of Medical Sciences were selected. Patients were randomly divided into two groups of 30 people. Patients in both groups continued their standard anti-diabetic therapy. In addition, one group was administered one 500 mg herbal capsule and the other group placebo capsule every 12 hours for three months. The blood biochemical tests including, fasting glucose, glycosylated hemoglobin, lipid profile, creatinine, urea and liver enzymes including SGOT, SGPT, ALP were performed at the baseline, and then, at the end of the study after 3 months. The results showed that the fasting blood glucose level and glycosylated hemoglobin in the patients treated with herbal combination decreased significantly after 3 months compared with the baseline and also the placebo ($p = 0.000$). No significant ($p > 0.05$) changes were observed in the blood lipid profile, liver enzymes and kidney function tests. No side effects were observed during the study in both groups. In conclusion, the present study showed that, consumption of this herbal combination for 3 months has anti-diabetic effect without any side effects.

Keywords: Persian Medicine, Medicinal plants, Type 2 diabetes, Clinical trial, Herbal combination

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INTRODUCTION

Diabetes is undoubtedly one of the highest prevalence, debilitating and costly chronic diseases in today's world with all the advances in the pharmaceutical and medical sciences. Although many drugs are available for the treatment of diabetes, the uncontrolled disease is still responsible for many health care problems such as blindness, impotence, diabetic wounds, amputation, kidney and cardiovascular diseases [1]. Therefore, research to find effective anti-diabetic drugs without adverse effects is on the agenda of the researchers. The field of medicinal plants, that has a long history of use by diabetic patients, is one of the research areas for anti-diabetic remedies.

In the Persian Medicine, herbalists prescribe many anti-diabetic medicinal plants, in formulations of single or combination of several different plants for the treatment of diabetes. One of the formulations used for the treatment of diabetes, is a combination of silymarin, lemon balm, Caucasian whortleberry, fenugreek, nettle, and colocynth. The anti-hyperglycemic effects of each herb in the combination have been reported in a number of experimental and clinical trial studies. Silymarin is a well-known hepatoprotective herb that has been reported in a number of studies to have anti-diabetic effects as well as prevention of chronic complications of diabetes [2-5]. Lemon balm is known for its psychoactive properties, and also its essential oil as well as

ethanolic and aqueous extracts have exhibited antioxidant and anti-hyperglycemic activities in diabetic animals [6-8]. Caucasian whortleberry has been used in the Persian Medicine to treat hypertension and diabetes. The anti-hyperglycemic and antioxidant effects of its fruit and leaf extracts have been reported in diabetic rats [9, 10]. Fenugreek seeds and leaves, another ingredient of this formulation, are used as food due to their high nutritional value, and also used as remedy to treat various diseases, especially diabetes. A number of studies have confirmed the effectiveness of its seed extract in improving most metabolic symptoms associated with both type 1 and type 2 diabetes [11, 12]. Nettle is another ingredient in this formulation which has been widely used by diabetic patients [13]. In an experimental animal study, nettle leaf extract showed a dose-dependent glucose-lowering effect persistent for up to 48 hours [14]. In another study, its anti-hyperglycemic effects have been reported in high-fructose-fed rats [15]. Colocynth fruit is also widely used by diabetic patients, especially those suffering from constipation [16]. The plant has many pharmacological effects including anti-diabetic, antioxidant, anti-inflammatory, and strong laxative effects which have been demonstrated in experimental studies [17, 18].

Considering that the anti-diabetic effects and safety of each plant in the herbal combination have been reported in the previous studies, the present clinical trial was conducted to investigate the safety and efficacy of this formulated herbal combination in type 2 diabetic patients.

MATERIALS AND METHODS

Plant Materials

The seeds of *Silybum marianum* and *Trigonella foenum-graecum* were purchased from the local market in Tehran city; aerial parts of *Mellisa officinalis* (IMPH code: 1494) were collected from the Arak province; fruits and leaves of *Vaccinium arctostaphylos* (IMPH code: 1439) from the Guilan province; aerial parts of *Urtica dioica* (IMPH code: 744) from the Ghazvin province and fruits of *Citrullus colocynthis* (IMPH code: 3601) were obtained from the local market from the Yazd city.

The plants and seeds were identified by Dr. Maryam Ahvazi (botanist) and voucher specimens of the plants were deposited in the Herbarium of the Institute of Medicinal Plants (IMPH), ACECR, Karaj, Iran.

Plant Extract

The plants materials were washed and dried in shade at room temperature. The dried plants material was crushed and extracted separately with 70% aqueous ethanol using percolation method at room temperature. The extracts were filtered through Whatman no. 1 filter paper and evaporated to dryness under reduced pressure at a maximum of 40 ◦C using a rotary evaporator instrument. The dry extracts were used for combination, granulation and finally encapsulation.

Herbal and Placebo Capsules Preparation

The 500 mg identical appearance capsules of the herbal combination (herbal capsules) and placebo (toasted flour) were formulated. The selection of the amount of each plant in the herbal combination and the patient's daily dosage were according to the method of preparation and daily dosage used in the Persian Medicine. Five to 10 g of this herbal combination in the form of tea or filled in capsules is used in two or three divided doses daily in the Persian Medicine as an anti-hyperglycemic agent. Accordingly, the daily dose of 1000 mg herbal combination in two divided doses (500 mg capsule) was selected in the present clinical study.

Standardization of the Herbal Combination

The herbal combination was standardized by quantitative determination of the total phenol and flavonoid contents [19, 20]. In addition, the main constituents of some major plants in the herbal combination including silymarin (silibinin), lemon balm (rosmarinic acid), nettle (chlorogenic acid) and fenugreek (trigonelline) were determined [21-24].

Ethical Considerations

The present randomized, double-blind, placebo-controlled clinical trial protocol was approved by the Medical Ethics Committee of the Ebne-Sina Institute (IR.ACECR.AVICENNA.REC.1398.013; date of approval: 11.04.1398), and registered in the Iranian Registry of Clinical Trials (IRCT20080901001157N15.) The clinical trial was conducted in the Diabetes Clinic of Imam Ali Hospital affiliated to the Alborz University of Medical Sciences. Written informed consent was obtained from each patient prior to the study.

Protocol

Patients

Sixty voluntary Iranian male and female patients from a total of 93 type 2 diabetes patients referred to the diabetes center affiliated with the Imam Ali Hospital of the Alborz University of Medical Sciences in Karaj, were included in the study. The patients were visited by an endocrinologist and enrolled in the study if they met the entry criteria.

Inclusion Criteria

Patients with type 2 diabetes with a healthy physical condition; aged 40 to 65 years; under standard anti-hyperglycemic drug therapy; with no change in drug doses in the past 2 months; fasting blood glucose 140-180 mg/dL and glycosylated hemoglobin (HbA1c) of 7-8.5%

Exclusion Criteria

Pregnant and lactating patients; patients on insulin; patients with serious heart, kidney, liver or hematological diseases; hypothyroidism and epilepsy patients.

Sample Size

The sample size was calculated at 30 patients in each group to estimate 10% difference of HbA1c as primary outcome between the two groups, considering type I error = 0.05, power of 80% and 10% dropout.

Interventions

Sixty eligible type 2 diabetic patients were randomly allocated to 2 parallel groups of 30 patients each. One group received the herbal capsule (500 mg) and the other group received a placebo capsule (500 mg) every 12 hours with food. All patients in both groups were advised to take their routine anti-hyperglycemic drugs during 3 months of the study without changing the dosage. The patients, medical staff and data analyzer were blind to the drug allocation. Blood biochemical parameters were determined at the baseline and 3 months after the intervention in both groups. The patients' blood samples were taken after a 12-hour overnight fast and sent to the hospital's central laboratory for analysis.

Outcomes

Fasting blood glucose and glycosylated hemoglobin levels were primary outcomes, and fasting total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein

cholesterol (HDL-C) and triglyceride (TG) levels were secondary outcomes.

Evaluation of the Herbal Capsule Safety and Adverse Effects

Fasting blood levels of serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), alkaline phosphatase (ALP), blood urea nitrogen (BUN) and creatinine (Cr) were determined. In addition, patients were in contact with their physician during the study to report any abnormal health events.

Statistical analysis

Paired and independent samples t, and chi-squared tests were used for statistical comparison of study outcomes between the herbal and placebo groups. The data were analyzed by the intention-to-treat method. *P* values less than 0.05 were considered significant.

RESULTS

Standardization of the Herbal Combination

The total phenolic content of dry herbal combination was 85.32 mg as gallic acid equivalent per gram and flavonoid content was 137.07 mg as rutin equivalents per gram. Further chemical analysis showed that one gram of herbal combination contained 3.3 mg silibinin, 8.1 mg rosmarinic acid, 7.8 mg chlorogenic acid and 5.3 mg trigonelline as main constituents of silymarin, lemon balm, nettle and fenugreek, respectively (Table 1).

The Enrollment and Baseline Characteristics Data

From June 2023 to March 2024, out of 93 type 2 diabetic patients referred to the diabetes center, 60 patients were randomly entered into the herbal and placebo groups. All patients, with the exception of 3, cooperated until the end of the study, but data of 60 patients were included in the analyses according to the intention-to-treat method. The CONSORT flowchart describing the progress of the patients through the trial is shown in Figure 1.

Table 1 Constituents of the herbal combination

No.	Constituents	mg/g of dry herbal combination
1	Total phenols	85.32
2	Flavonoids	137.07
3	Silibinin	3.3
4	Rosmarinic acid	8.1
5	Chlorogenic acid	7.8
6	Trigonelline	5.3

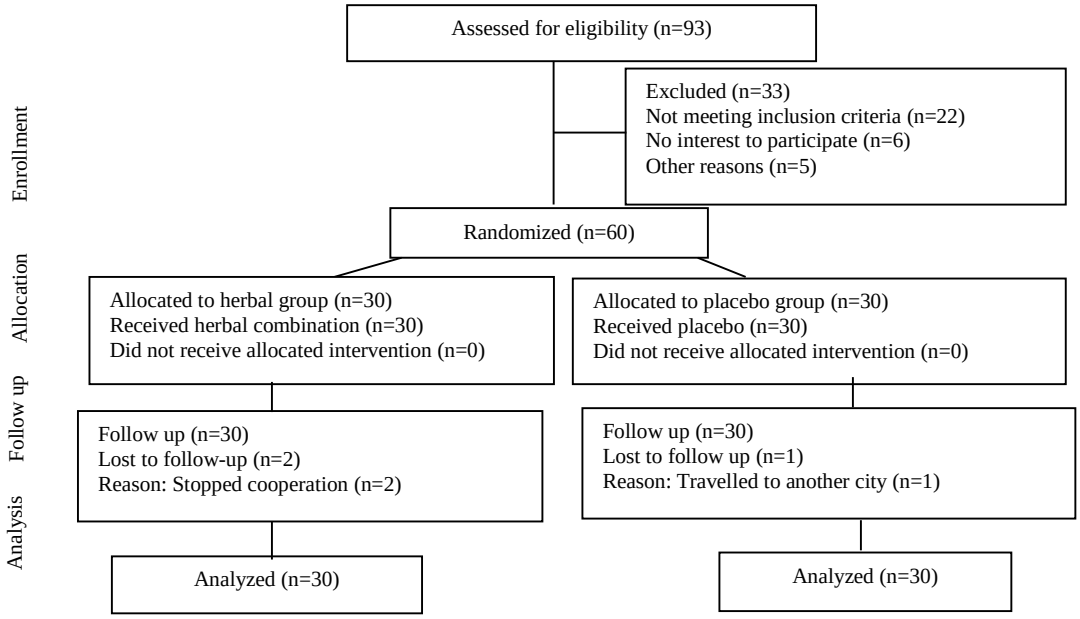

Fig. 1 CONSORT diagram showing the flow of participants from enrolment up to the end of the study

Table 2 Baseline characteristics of the patients in two groups

Parameter	Herbal group	Placebo group	*P*
Age (years)	57.4 ± 7.4	55.3 ± 6.0	0.173 *
Gender (male/female)	40% (20) / 60% (30)	46% (23) /54% (27)	0.163 **
Duration of disease (years)	7.5 ± 5.9	6.0 ± 4.5	0.059 ***

**P*- value based on independent samples *t*- test

***P*- value based on chi-squared test

****P*- value based on Mann–Whitney U test

Values are presented as mean ± standard deviation, where appropriate

$P < 0.05$ was considered statistically significant (paired t and chi-squared tests)

Intervention Outcomes

The laboratory glycemic and lipid profile data are given in the Table 3. The data analyses showed significant decrease of FBS and HbA1C at the end of the study in the herbal group compared with the baseline ($P < 0.001$ and $P < 0.001$, respectively). Furthermore, statistically significant decrease was observed in the FBS and HbA1C in the herbal group compared with the placebo group at the end of the study ($P < 0.001$ and $P < 0.001$, respectively). The percentages of endpoint reductions of FBS and HbA1c levels are also presented in the Table 3. In addition, data analysis showed that no statistically significant differences were observed for lipid profile at the endpoint between groups and also compared with the baseline. As presented in Table 2 the basic characteristics of patients including the mean age, gender, and duration of diabetes were not significantly different between the two groups.

Table 3 Glycemic and lipid values at the baseline and endpoint in the herbal and placebo groups

		Placebo group Mean ± SD	Herbal group Mean ± SD	*P*-value between groups	Percent change between groups
FBS (mg/dL)	Baseline	160.0 ± 31.6	156.6 ± 34.3	0.683	
	Endpoint	162.1 ± 25.1	132.5 ± 26.8	0.000	16.71 ↓
	p	0.664	0.000		
HbA1c (%)	Baseline	8.2 ± 0.7	7.9 ± 1.1	0.220	
	Endpoint	8.4 ± 0.7	6.9 ± 1.0	0.000	15.0 ↓
	p	0.443	0.000		
TG (mg/dL)	Baseline	155.6 ± 63.2	161.1 ± 61.7	0.719	
	Endpoint	148.0 ± 52.2	163.5 ± 52.9	0.281	3.3↑
	p	0.363	0.790		
TC (mg/dL)	Baseline	180.3 ± 34.0	173.0 ± 42.0	0.490	
	Endpoint	173.6 ± 34.2	162.9 ± 45.5	0.482	2.12 ↓
	p	0.159	0.159		
HDL-C (mg/dL)	Baseline	44.3 ± 6.6	42.1 ± 8.5	0.551	
	Endpoint	42.1 ± 6.3	42.9 ± 11.1	0.924	2.16 ↓
	p	0.104	0.626		
LDL-C (mg/dL)	Baseline	97.3 ± 26.2	92.7 ± 29.7	0.443	
	Endpoint	94.5 ± 26.6	88.3 ± 27.8	0.542	1.83 ↓
	P	0.535	0.106		

FBG: Fasting blood glucose; HbA1c: Glycated hemoglobin; TG: Triglyceride; TC: Total cholesterol; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol; SD: Standard deviation

**P*-value by paired and unpaired t-tests

$P < 0.05$ was considered statistically significant

Herbal Capsule Safety and Adverse Effects

Data analysis showed that there was no statistically significant difference in the kidney and liver blood parameters at the end of the study between the two groups, indicating the safety of the herbal combination (Table 4). In addition, the patients did not report any side effects during the study, except for 3 patients in the herbal group who complained of intestinal cramps, which subsided after temporarily reducing the dose of the herbal combination.

Table 4 Liver and kidney parameters levels at the baseline and endpoint in the herbal and placebo groups

Parameter	Analysis	Herbal group Mean ± SD	Placebo group Mean ± SD	*P*-value between groups	Percent change between groups
SGPT (U/L)	Baseline	28.4 ± 15.1	30.4 ± 15.4	0.203	
	Endpoint	24.5 ± 15.5	27.1 ± 10.4	0.224	1.25↓
	p	0.167	0.545		
SGOT (U/L)	Baseline	25.8 ± 9.6	29.5 ± 14.6	0.084	
	Endpoint	23.5 ± 8.3	26.1 ± 11.6	0.618	1.1↓
	p	0.618	0.420		
ALP (U/L)	Baseline	138.2 ± 17.5	140.2 ± 19.5	0.536	
	Endpoint	140.6 ± 13.7	144.7 ± 16.9	0.311	1.25↑
	p	0.344	0.185		
BUN (mg/dL)	Baseline	17.4 ± 7.3	19.6 ± 4.3	0.056	
	Endpoint	16.5 ± 5.6	19.9 ± 5.2	0.067	3.5↓
	p	0.150	0.790		
Cr (mg/dL)	Baseline	0.98 ± 0.21	0.95 ± 0.12	0.847	
	Endpoint	0.97 ± 0.24	0.97 ± 0.12	0.782	1.06↓
	p	0.133	0.103		

BUN: Blood urea nitrogen; Cr: Creatinine; SGOT: Serum glutamic-oxaloacetic transaminase; SGPT: Serum glutamic-pyruvic transaminase; ALP: Alkaline phosphatase; SD: Standard deviation

**P* -value by paired and unpaired t-tests

$P < 0.05$ was considered statistically significant

DISCUSSION

In the present study, the anti-diabetic potential of herbal combinations containing silymarin, lemon balm, nettle, fenugreek, nettle, Caucasian whortleberry, and colocynth plants was investigated in type 2 diabetic patients. In this 3-month clinical trial, the administration of the herbal combination to type 2 diabetic patients at a dose of 500 mg twice a day caused anti-hyperglycemic effects as significant decreases in the fasting blood glucose and HbA1c levels. The lack of significant effect on the blood levels of creatinine, BUN, SGOT, SGPT, and ALP at the end of the study shows that the herbal combination has no toxic effects on the liver and kidney functions. The anti-hyperglycemic effects observed in the present study are consistent with the previous reports showing that each herb in this combination has anti-diabetic effects in clinical trials in addition to several experimental studies. In a number of clinical trials, anti-hyperglycemic effects of silymarin at a daily dose of 600 mg have been reported in type 2 diabetic patients [2, 25], type II diabetic patients candidate for insulin therapy [26], and first-degree relatives of type 2 diabetic patients [27]. Likewise, the anti-diabetic effect of lemon balm leaf extract with a daily dose of 500 mg has been reported in a clinical trial study on type 2 diabetic patients [28]. The anti-hyperglycemic effects of Caucasian whortleberry fruit extract 1050 mg daily for 2 months were reported in type 2 diabetic patients [29, 30]. The results of several clinical trials support the beneficial effects of fenugreek seed extract 2000 mg per day as an adjunctive therapy in the control of blood glucose in patients with type 2 diabetes [31-33]. The anti-

hyperglycemic effects of nettle have been demonstrated in a clinical trial with the daily consumption of 1500 mg of its leaf extract in patients with advanced type 2 diabetes [34]. Also, in two other studies, a significant improvement in insulin resistance indices was reported following treatment with nettle leaf extract in type 2 diabetic patients [35, 36]. The blood glucose-lowering effect of colocynth has been demonstrated in a clinical trial by administering 300 mg of its fruit powder daily to patients with type 2 diabetes [37].

Several mechanisms have been proposed for the anti-diabetic activities of the plants in this herbal combination. Alpha-amylase and alpha-glucosidase are two key enzymes in the gut for the final stages of carbohydrate digestion, and inhibiting them in the gut reduces the amount of glucose available to enter the bloodstream [38]. Inhibition of α-amylase and α-glucosidase activity by each of the plants in this herbal combination is important anti-diabetic mechanism reported [39-44]. Insulin resistance or impaired insulin sensitivity is another important defect in all types of diabetes, which is reflected by reduced glucose uptake into skeletal muscles, liver, and adipose tissue [45, 46]. However, favorable effects on insulin resistance or insulin secretion are other important anti-diabetic mechanisms reported for the herbs in this herbal combination [6, 47-49]. Defects in the intestinal microbiota is another factor that is related to the development of type 2 diabetes [50]. However, most of the plants in this herbal combination modulate the gut microbiota in favor of diabetes improvement [51-54]. Strong evidence suggests that oxidative stress and inflammation are associated with the risk of type 2 diabetes and its associated complications through various mechanisms including oxidative β-cell dysfunction and insulin resistance [55]. In this regard, in support of the therapeutic effects observed in the present study, antioxidant effect has been reported for each plant present in this herbal combination [25, 56-60]. Worthy to note, short treatment period and small sample size are limitations of this study.

CONCLUSION

The present study showed that consumption of the herbal combination containing silymarin, lemon balm, Caucasian whortleberry, fenugreek, nettle, and colocynth with a dose of 500 mg twice a day improves hyperglycemia in type 2 diabetic patients. No side effects were observed during the three months of the study. In order to obtain maximum anti-diabetic efficacy with no side effects, it is suggested to conduct more clinical trial studies using combination of different doses of these plants in the future.

Author Contributions

The work presented in this article was carried out through collaboration between the following authors: H.F.H.: made the initial hypothesis and designed research. S.K. and R.M.S.: collected data. M.A.: Collected and identified the plants and revised and approved the final draft. B.T.: analyzed data. H.P., N.R., S.B., and R.M.: supervised and conducted the clinical trial. All authors have read and approved the final manuscript. All data were generated in-house and no paper mill was used.

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Abbreviations List

FBG: Fasting blood glucose; HbA1c: Glycated hemoglobin; TG: Triglyceride; TC: Total cholesterol; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol; BUN: Blood urea nitrogen; Cr: Creatinine; SGOT: Serum glutamic-oxaloacetic transaminase; SGPT: Serum glutamic-pyruvic transaminase; ALP: Alkaline phosphatase; SD: Standard deviation; IMPH: Institute of Medicinal Plants Herbarium

Conflict of Interest

The authors declare no conflict of interest.

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